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“PARFUM COMPONENTEN: CONTACT ALLERGIE EN ALLERGISCHE CONTACT DERMATITIS”

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“FRAGRANCES: CONTACT ALLERGY AND ALLERGIC CONTACT DERMATITIS”

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ABBREVIATIONS

ACD Allergic Contact Dermatitis

ICD Irritant Contact Dermatitis

Th T-helper

MHC Major Histocompatibility Complex

IL interleukin

TCR T-cell receptor

CYPs cytochrome P450-dependent mono-oxygenases

SAR structure activity relationships

APCs antigen-presenting cells

FM I fragrance mix I

FM II fragrance mix II

MP *Myroxylon pereirae*

IFRA International Fragrance Association

HICC (*hydroxyisohexyl 3-cyclohexene carboxaldehyde*)

IDVK German Information Network of Departments of Dermatology

BHT butylated hydroxytoluene

YY ylang-ylang

ICDRG International Contact Dermatitis Research Group

SCCP Scientific Committee on Cosmetics Products

OR odd ratio

CI confidence interval

Chapter 1

Introduction

GENERAL INTRODUCTION

Adverse reactions to fragrances include allergic contact dermatitis (ACD), irritant contact dermatitis (ICD), photosensitivity, immediate contact reactions (contact urticaria), pigmented contact dermatitis, and also (worsening of) respiratory problems (de Groot, 2001). Contact allergy to fragrances, being a delayed hypersensitivity response that may clinically result in ACD is a common finding. It is due to a combination of repeated environmental exposure and age-related susceptibility factors (Buckley et al, 2003) and is commonly observed in patch-test populations, particularly, since allergenic fragrance chemicals are ubiquitous in our environment, not only in fine fragrances but also in a wide range of cosmetic, household and industrial products (Matura et al, 2003). Actually, together with preservative agents, they are the most important sensitizing culprits in cosmetic products (Goossens, 2006-Schnuch et al, 2004). In this introductory chapter we will shortly review the chemistry, characteristics and the potential to induce adverse reactions.

I. GENERAL ASPECTS ON FRAGRANCES

1.1. Fragrance chemicals

Perfumers have been creating fragrances for approximately 3.000 years. Over the centuries the role of fragrance in society has gained in importance, aiming to improve the quality of life by underlining the personality, attractiveness and well-being of its bearers. Today, the occupation as a perfumer is a well-established, but rather rare profession. The prerequisites for this profession include skills, such as memory for odors and their characteristics, as well as an artistic talent (Harder, 1998).

A perfume is a blend of odorous ingredients composed of a diluent, usually ethanol, and a mixture of 10 to 300 different fragrance ingredients. Most fragrance chemicals are volatile aliphatic or aromatic molecules that can be categorized according to their functional groups, including aldehydes, alcohols, ketones, acids, esters, ethers, terpenes, etc. The most volatile ingredients are called “top notes” (citrus, green-leafy, herbaceous, fruity and spicy). The most essential part of the perfume is formed by the “bouquet” or “heart note” built up by floral accords. The long-lasting materials are known as “bottom” or “dry-down” notes and include woody, moss-like and sweet vanilla-like ingredients together with animal elements, mostly of musky character. Approximately 3000 different fragrance chemicals are currently used in the perfume industry and are often combined to create characteristic scents (Ford, 1991).

1.2. Exposure

Perfumes and virtually all cosmetics and toiletries such as deodorants, aftershaves, skin care products, lipsticks, powders, shampoos and soaps contain fragrance chemicals. In addition, they are

present in various household products such as fabric softeners, detergents, cleansing agents, polishes and air fresheners, as well as in industrial products such as soluble oils and degreasers. Moreover, several products or materials such as cars, candles, toys, and various branded articles may be perfume scented.

Fragrance chemicals may come in contact with the skin, eyes, or respiratory organs.

With regard to skin contact, any part of the body is exposed, i.e. scalp: shampoo, hair lacquer, hair gel; face: skin care products, aftershave, perfumed tissue handkerchiefs, airborne from perfumes on clothing; the eyelids: eye cosmetics; the lips: lipstick, toothpaste; the neck: aftershave, perfume; the trunk: body-care products; the axillae: deodorants; the perianal area: fragranced (moistened) toilet tissues or wipes; the vulvar area: feminine hygiene sprays, sanitary napkins, topical drugs; the hands: moisturizing creams, soaps, and, in fact, any scented product; the feet: antiperspirants or skin care products.

The contact may be intentional, e.g. by direct contact with perfumed cosmetics and medications, or unintentionally, by evaporation from consumer products or from other persons wearing perfume. Moreover, fragrances also occur in an occupational environment, i.e. workers in the cosmetics industry, such as beauticians, hairdressers, and aroma therapists, housewives, health-care, and cleaning personnel, as well as in industrial settings. Thus, it can be stated that fragrance materials are ubiquitous and it is estimated that 95% of the female population and at least 75% of the male population come into daily contact with cosmetic products (Fisher, 1980-Nielsen et al, 1993).

1.3. Adverse effects from fragrances

The ubiquitous presence of fragrances in modern society coupled with the increased usage of fragrance-containing products by people of raising economic power (Lunder et al, 2000), as well as higher interest in 'all natural' products, which sometimes contain fragrance sensitizers in high enough concentrations to induce sensitization (Somogyi, 1996), all contribute to the increase in adverse effects observed. They may be categorized according to localization (i.e. the skin or respiratory organs) or pathophysiology (i.e. type of immunological response) (Rastogi et al, 1996-de Groot et al, 1997-Eriksson et al, 1987).

1.3.1 Respiratory and other mucosal symptoms

The adverse effects of fragrance chemicals related to the respiratory organs have not been well described and the underlying pathophysiology of the symptoms is unclear. In a single case, anaphylaxis has been reported after spraying perfume in the eyes (Lessenger, 2001), which could indicate mechanisms associated with IgE-mediated allergy. Studies in selected groups of patients

with lower respiratory symptoms have shown that both inhalation and exposure of only the eyes to vapors of perfume may elicit lower respiratory symptoms, in addition to eye symptoms (Eriksson et al, 1987).

A decline in lung function after perfume inhalation has been reported in individuals with severe asthma (Jensen et al, 1991), which has also been observed in subjects with occupational perfume exposure (Millqvist et al, 1996-Millqvist et al 2001-Schnuch et al 2002-Frosch et al, 2002-Baur et al, 1999). It has, however, been disputed whether all the respiratory symptoms elicited by fragrance products in persons with asthma can be attributed to bronchial obstruction (Millqvist et al, 1998). Moreover, lower respiratory symptoms associated with them are also frequent among non-asthmatic and non-allergic individuals (Kumar et al, 1995). The mechanisms involved could be similar to those observed with inhalation of capsaicin (the pungent principle in hot chili pepper), an experimentally used material to evaluate respiratory response, which stimulates the afferent C-fibres and Aδ-, fibres in the airways (Belvisi, 2003), and triggers the cough reflex in a dose-dependent way in normal individuals (Midgren et al, 1992).

Elberling et al. (2005a- Elberling et al, 2005b-Elberling et al, 2004ab, Elberling et al, 2006) concluded that the association between eye and airway symptoms elicited by airborne chemicals may represent a syndrome, which is related to allergic contact dermatitis and perhaps hand eczema, but not to IgE-mediated allergy to proteins. Moreover, although psychological factors play a significant role in reporting symptoms elicited by airborne chemicals, both endogenous and environmental factors may be of greater importance for the induction and elicitation.

In more recent studies, Schnuch *et. al* (2010) have shown that the lung function as expressed by forced expiratory volume in 1s (FEV1) did not change after any fragrance exposure. However, inhalation of fragrance compounds in high concentrations resulted in systemic (haematogenic) allergic contact dermatitis (flare) and increased skin symptom scores 24 and 72h following exposure in single patients allergic to 'the' fragrance. They concluded that inhalation of higher doses of potent contact allergens should be avoided by subjects specifically sensitized to these allergens.

1.3.2. Dermatological problems

Fragrance components can cause a number of local (and systemic) reactions. The following are the most common:

- Contact dermatitis- allergic and irritant
- Photosensitivity- allergic and toxic
- Urticaria, both immunological and non-immunological
- Pigmentation and depigmentation

The most common skin reaction seen by dermatologists to fragrance materials is allergic contact dermatitis. Most clinical pictures concern erythematous lesions at the contact site, but some cases may resemble nummular eczema, seborrhoeic or atopic dermatitis (especially in the skin folds), sycosis barbae, or lupus erythematosus (Meynadier et al, 1986). More acute lesions with papules, vesicles and oozing may sometimes be observed as well. With regard to the localization of the lesions, it can be expected that the neck, the skin behind the ear and the axillae are often implicated, given that they are exposed to products with high concentrations of fragrances (perfume, deodorant). Moreover, the sensitive skin of the face and eyelids is particularly susceptible to develop ACD from fragranced skin-care products, decorative cosmetics and cleansing preparations, and also from airborne exposure (Dooms-Goossens, 1993a-b); in man micro-traumata from shaving may facilitate skin penetration, thus contact and also photo-contact sensitization, the latter in combination with U.V. exposure. Moreover, also hand eczema is common in fragrance-sensitive patients (Edman et al, 1994, Johansen et al, 1996, Heydorn et al, 2003 a-b); usually, they first develop irritant dermatitis or atopic dermatitis, which is later complicated by contact allergy to products used for treatment (fragranced topical drugs) or prevention (hand creams and lotions), or to other perfumed products contacted in the household, hobby, or work environment. Dyshidrotic eruptions on the hands have been attributed to ingestion of spices (Meynadier et al, 1986). Besides atopy, other existing eczematous conditions, located in the perianal or vulvar area (Nardelli et al, 2004) may be complicated by fragrance allergy, and even facial psoriasis may be induced/ aggravated by ACD from fragrances (de Groot et al, 1983).

Regarding photo-allergic dermatitis, during the 1980s numerous cases were reported due to musk ambrette (Raugi et al, 1979) and 6- methyl coumarin (Jackson et al, 1980), the former primarily observed in men due to after shave lotions, the latter associated with high concentrations used in a certain fragranced sunscreen. Both of these chemicals were removed from the market, hence such reactions have not been observed since.

In this context we will focus on ACD.

Chapter 2

Contact Allergy and the main fragrance allergens

The frequency of contact allergy to perfumes is estimated to be 1%-2% in the general population (1.1 % Denmark, 1.8% Norway) (Nielsen et al, 1992-Dotterud et al, 2007), and roughly 8% in contact eczema patients (de Groot, 2001-Buckley et al, 2003-Mortz et al, 2013). Of course, not all patient with contact allergy do actually suffer from allergic contact dermatitis (ACD).

2.1 Mechanism of allergic contact dermatitis

Allergic contact dermatitis (ACD) is the clinical manifestation of contact allergy, a delayed-type hypersensitivity (type IV immunological reaction) (Rustemeyer et al, 2001). The immune response in ACD is divided in two steps: *Sensitization Phase* also called afferent or induction phase. This step lasts 8 to 15 days in humans and generally has no clinical consequences. On the other side the *Effectors Phase* or *Elicitation Phase* is produced within 3 days with clinical manifestations of an inflammatory reaction, i.e. eczema, which persists over several days and progressively decreases upon physiological down-regulatory mechanisms (Figure 1). The innate immune mechanisms and the skin immune systems involved in contact dermatitis will not be discussed in detail in this manuscript; only the concepts of haptens, "pre- and pro-haptens" will be mentioned because some fragrance allergens behave as the latter.

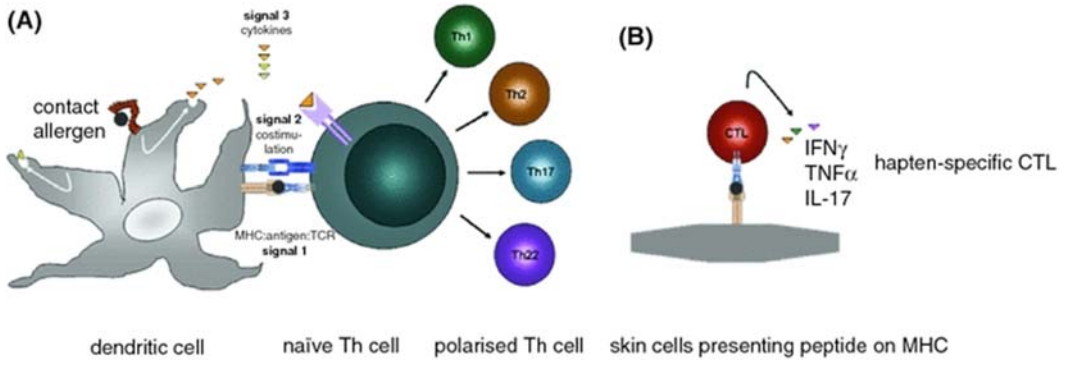

Figure 1

Lymphocyte-mediated immune mechanisms in contact allergy. Sensitization phase (**a**). The contact allergen activates dendritic cells in the skin via 'pattern recognition receptors' such as TLRs. Subsequently naïve T helper (Th) cells are polarized upon specific recognition of the haptenated allergen by the major histocompatibility complex (MHC), co-stimulatory signals and cytokines such as IL-12, IL-4, IL-1β and IL-6. Elicitation phase (**b**). Hapten-specific cytotoxic $CD8^+$ T lymphocytes (CTLs) release inflammatory cytokines and induce disease-specific local skin lesions following re-exposure of the skin to the same contact allergen (Peiser et al, 2012) (reprinted with permission by Springer).

2.1.1 Haptens, pro- and prehaptens

Contact allergy caused by low molecular weight compounds (so-called haptens) requires the formation of antigenic hapten-protein complexes. The potential of a low molecular weight compound to become an allergen is determined by its ability to penetrate the skin, to react with skin proteins, and its processing and presentation by dendritic cells to memory T-lymphocytes. Some compounds will react directly (e.g. nickel), while others require activation, either externally or metabolically inside the skin (Karlberg et al, 2008). Prehaptens are compounds with no or low sensitizing potential that are activated externally, while prohaptens have been defined as non-sensitizing compounds that require metabolic activation (Lepoittevin, 2006).

Examples of prehaptens are found among the unsaturated hydrocarbons and ethers such as common fragrance terpenes, diterpenes in colophonium and ethoxylated surfactants. Patch tests revealed some of these substances to be potent skin sensitizers following their activation by autoxidation. For example, whereas limonene and linalool, two frequently used fragrance components, rarely cause sensitization by themselves (Christensson et al, 2010-Matura et al, 2002-2005-Brared Christensson et al, 2009-2012-Schnuch et al, 2007), autoxidation results in the formation of the corresponding hydroperoxides (Skold et al, 2008), for which multicentre studies have shown that they are among the most common causes for ACD (see below).

With regard to prohaptens, their activation could vary depending on the individuals' enzymatic expression patterns. Well-known examples of prohaptens are cinnamyl alcohol (3-phenyl-2-propen-1-ol) and urushiols (Kalergis et al, 1997-Elahi et al, 2004).

Some compounds act both as prehaptens and prohaptens and, depending on the way of activation, the resulting haptens can have a different skin sensitization potential. Well-studied examples are cinnamyl alcohol (see below) and the moderate sensitizer geraniol, which is present in fragrance mix (I) in the baseline series for the diagnosis of contact allergy. Studies showed geraniol to act as a prehapten and prohapten activated by cytochrome P450-dependent mono-oxygenases (CYPs), both leading to the formation of geranial and neral; beside, enzymatic activation also produces sensitizing epoxides (6,7-epoxygeraniol and 6,7-epoxigeranial), while autoxidation results in the formation of sensitizing hydroperoxides (Hagvall et al, 2007-2008-2012).

Considering the importance of oxidation for the formation of haptens, autoxidation and CYP-mediated metabolism should be part of the hazard identification for potential contact allergens. This can be achieved by predicting autoxidation using structure activity relationships (SAR) and by in-vitro CYP activity assays. A recently developed CYP cocktail is based on cutaneous CYP enzymes

and thus allows studying part of the skin metabolism in vitro (Peiser et al, 2012). Furthermore, diagnosis of contact allergens should include patch tests with oxidized forms of the corresponding substances.

2.2 Fragrances as complex mixtures of molecules

Sensitization to even a simple mixture of molecules with very different physicochemical properties results in many interactions during skin penetration, metabolism and epitope formation by reaction with the nucleophilic residues of proteins. Not all mixes are the same; two main categories can be distinguished (Dupuis et al, 1982), i.e. with similar structures (homogenous mixes) and those consisting of a mixture of molecules (heterogeneous mixes) that are chemically and structurally unrelated.

2.2.1 *Homogenous Mixes*

This category consists of a mixture of molecules that are very similar in structure and reactivity. The factors controlling molecular recognition during the elicitation phase of the allergic response are highly dependent on the chemical group and the spatial geometry of the molecule. All the molecules have the same chemical reactivity, are metabolized in the same way and have analogous physicochemical properties.

2.2.2 *Heterogeneous Mixes*

This category is the most challenging for the chemist, since the presence, in the mixtures, of several molecules which differ widely in size and reactivity, can result in many interactions during skin penetration, bio-distribution of molecules between the various skin compartments, during metabolism, or with antigen-presenting cells.

Perfumes fall into this complex category, as do the test materials to identify fragrance allergy (see below), with all problems inherent in the use of molecular mixtures, either during the sensitization or challenge phases. Indeed, fragrance allergens are not inert entities, but are liable to undergo modifications and/ or interactions with their surroundings, forming new compounds and leading to increased (synergistic) or decreased (antagonistic) allergenic reactivity.

With regard to the diagnosis of contact allergy to fragrances, mixtures or impurities make the interpretation of patch-test results very difficult and have been the cause of many substances being classified as sensitizing agents, when the allergen was, in fact, a degradation product or impurity. Testing with mixtures of compounds is thus an important source of error. These effects are, as yet, poorly quantified, but deserve all our attention, since the prevention policy currently in use in

perfume industry, which takes the allergenic potential of isolated molecules into consideration, is clearly limited, and the increasing number of cases of sensitization to perfumes should prompt us to look at the perfume as a whole (Lepoittevin et al, 1998-Bonefeld et al, 2011), Figure 2.

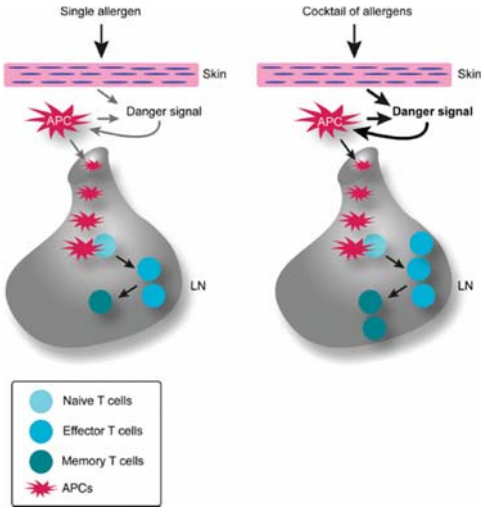

Figure 2 Model for the cocktail effect of fragrance allergens. Exposure to a single allergen induces only weak danger signals, and thereby weak activation of antigen-presenting cells (APCs), T cell activation, and memory T cell generation. However, exposure to a cocktail of allergens leads to enhanced danger signals, and thereby to a higher degree of APC and T cell activation and memory T cell generation. LN, lymph. (Bonefeld et al, 2011) (reprinted with permission by Wiley).

2.3 Identification of fragrance allergens. Diagnostic markers

Patch testing is a well-established method of diagnosing contact allergy. It aims to reproduce an eczematous reaction by applying allergens under occlusion on the intact skin of patients suspected to be sensitized.

The allergen to be tested is diluted in a vehicle, most often white petrolatum, and applied on the skin in a test chamber for 2 days. The preferred test site is the upper back. The general principle is to use the highest concentration of a test material that does not provoke any irritation in order to avoid false-positive reactions; however, also false-negative reactions due to a too low concentration of the test material may occur (Lachapelle et al, 2012-Mowitz et al, 2012). Patients with a suspected contact allergy are tested with a baseline series of common allergens, sometimes together with additional allergens specific for each individual case. Patch-test reading is carried out twice, in most dermatology clinics on day 2-3, and again on day 4-7. The reactions are scored according to their morphological characteristics as – (negative), ?+ (doubtful reaction), + (weak positive reaction), ++ (strong positive reaction), +++ (extreme positive reaction), or IR (irritant reaction), as recommended by the ICDRG (Wahlberg et al, 2001).

The main tool for diagnosing contact allergy to fragrances included in the European baseline patch-test series consists of fragrance mix I (FM I), *Myroxylon pereirae* (MP) or balsam of Peru,

and colophonium. More recently a second fragrance mix, i.e. FM II, was officially introduced as an additional marker in the baseline series in 2008 (Bruze et al, 2008).

2.3.1 Fragrance Mix I

FM I, or perfume mix I, was introduced for screening by Larsen (Larsen, 1977) in 1977 and concerns a heterogeneous mixture of seven individual components of varied nature and reactivity. It contains a naturally occurring plant material, i.e. *Evernia prunastri* (oak moss absolute) and six synthetic fragrances, i.e. isoeugenol, cinnamal, cinnamyl alcohol, hydroxycitronellal, eugenol, geraniol and α-amyl cinnamal (FM I 8% in petrolatum and each component of the mixture at 1% with sorbitan sesquioleate as an emulsifier). It has been included in the baseline series for many years. Compared to certain preparations, which contain more than 300 different molecules, it is a very simple "perfume" (Lepoittevin et al, 1998), but it can be used to illustrate the different aspects of, and problems inherent to molecular mixtures.

Indeed, the fragrance mix (Fig. 3), as well as other natural products such as *Myroxylon pereirae*, colophonium, and essential oils must be considered as a whole, rather than simply as the sum of its components, a point clearly made by the experience of many clinicians. Certainly, a positive response to the mix is not always accompanied by a positive result to one, or more of the constituents tested in isolation, which indicates that the mixture has a marked synergistic effect. And it is highly probable that this observation, made during testing (challenge), applies equally well to the sensitization stage.

As simple chemicals, FM I contains three large terpene 'families':

1. Three cinnamic derivatives
2. Eugenol and isoeugenol
3. Two linear monoterpenes (hydroxycitronellal and geraniol).

These three families of molecules differ greatly, not only in their structure, but also in their way of sensitization.

Figure 3. Fragrance-mix positive patch test.

Cinnamic derivatives

The three derivatives in the mix have different sensitization profiles: cinnamal and α-amylcinnamal can be considered as haptens, whereas cinnamyl alcohol, beside being a hapten (Bickers et al, 2005), was considered a pro-hapten, that can be partially metabolised to cinnamal. However, in addition, a recent study showed that cinnamyl alcohol autoxidizes rapidly upon air exposure, forming a highly allergenic expoxide and cinnamal, hence it also acts a prehapten (Niklasson et al, 2013). The joint presence of cinnamyl alcohol and cinnamal can result in an enzyme reaction in equilibrium (Figure 4), which is displaced as a function of the respective amounts of the two molecules.

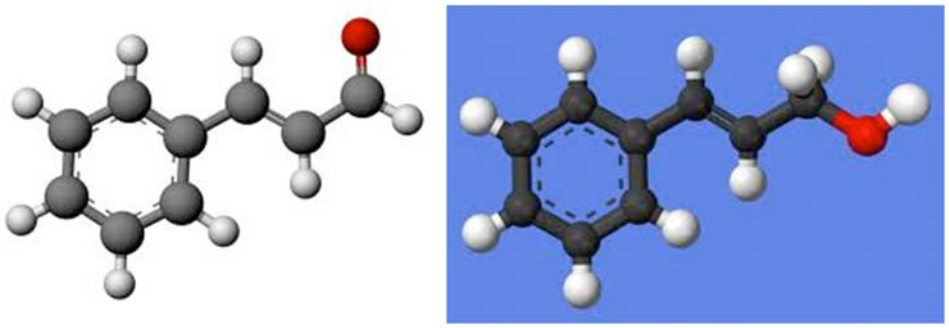

Figure 4. Cinnamal and cinnamyl alcohol

Eugenol and isoeugenol

Both molecules must undergo a metabolic oxidation step (in this case, a more complex one than with cinnamyl alcohol) to form the reactive hapten. Recent studies on the mechanism of activation of both molecules seem to indicate that they follow different metabolic pathways, explaining the observed difference in reactivity and the low degree of allergic cross-reaction between them (Bertrand et al, 1997-Johansen et al, 1997). Eugenol, the weaker sensitizing agent, seems to require an initial demethylation step, forming a catechol, which is then oxidized to the highly reactive ortho-quinone (Figure 5); on the other hand, isoeugenol is itself sufficiently reactive to be directly oxidized to the equally highly reactive paraquinone methide (Figure 6).

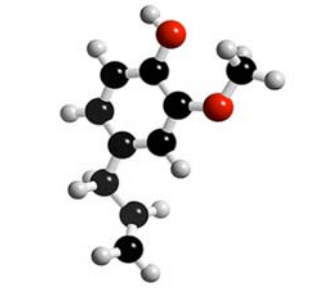

Figure 5. Eugenol

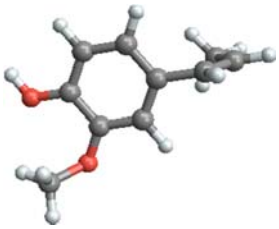

Figure 6. Isoeugenol

Moreover, also other derivatives such as trans-isoeugenol and isoeugenol-esters do react in patients sensitized to the parent compounds (Figure 7). Indeed, ester derivatives, such as eugenyl- and isoeugenyl acetate that are also used as fragrance ingredients (Tanaka et al, 2004), do cross-react with the alcohols, since they are hydrolyzed by esterases in the skin.

Figure 7.Positive patch test reactions to isoeugenol derivatives in isoeugenol-sensitive patients

Linear Monoterpene derivatives

This concerns geraniol, which is oxidized to the aldehyde, and hydroxycitronnellal, an aldehyde hapten. Again, the problem of the joint presence of an alcohol and aldehyde of related structure arises, together with the possibility of altered enzymatic metabolism (Hagvall et al, 2008-2012).

As previously said, geraniol acts both as a pro-hapten and pre-hapten. Testing with higher concentrations of oxidized geraniol and pure geraniol detects more patients than pure geraniol alone; but patch testing with only oxidized geraniol does not detect all cases of contact allergy to geraniol either. This indicates that other compounds formed may be important haptens as well. (Hagvall et al, 2013).

Evernia prunastri (Oak moss)

Oak moss absolute has been among the most frequent sensitizing ingredients in FM I, with positive reactions in 2.2-3.4% of patch-tested eczema patients (Hendricks et al, 1999-Frosch et al, 1995). Botanically, oak moss is the lichen, *Evernia prunastri,* which grows primarily on oak trees (Johansen et al, 2002). It is collected all over central and southern Europe, particularly in the former Yugoslavia and in France, but also in Morocco and Algeria. It has been considered the finest raw material for the production of perfume extracts.

Oak moss contains a large number of ingredients, including atranalol and chloroatranalol, which are among the most allergenic substances ever identified (Johansen et al, 2002), and this at extremely low concentrations, i.e. 0.1 p.p.m. In addition, it was shown that polymer-based treatment of oak moss extract reduces the allergenic elicitation potential in previously sensitized individuals only to a minor extent. Therefore, it must be considered that the residual concentrations of atranol and chloroatranol being less than 75 p.p.m. and 25 p.p.m., respectively, are still far too high to be safe regarding elicitation (Nardelli et al, 2009).

In the past, it has also been shown that the oak moss absolute used by the perfume industry as well as some patch-test materials contained resin acids, which were typically of another lichen extract, i.e. tree moss (Lepoittevin et al, 2000). Indeed, the fragrance industry often used mixtures of oak moss and tree moss, in order to improve the odor quality and to reduce costs, since the extracts from tree moss, *Evernia furfuracea*, are much cheaper than those from oak moss (Johansen et al, 2002). Tree moss is produced from a mixture of lichens growing on pine trees and small branches or pieces of bark may be present in the raw material, being most probably the source of the resin acids. The admixture of tree moss to oak moss may occur already at the time of harvesting and in this way the contamination of oak moss with tree moss resins acids may occur both unintentionally and deliberately. This means that, with regard to the diagnosis, contamination of oak moss patch test material with tree moss extract and thus (oxidized) resin acids, i.e. dehydroabietic and abietic acids, can lead to misdiagnosis, as these substances, or rather their oxidization products, are known allergens present in colophonium (Lepoittevin et al, 2000), with which it often co-reacts.

2.3.2 Myroxylon pereirae (balsam of Peru)

The natural product *Myroxylon pereirae* (MP) (balsam of Peru) is also used as a diagnostic marker for fragrance allergy in the European baseline series. It originates from a tree which grows in Central America and has been used as a fragrance ingredient due to its odorous properties. In fact, the crude Peru Balsam is not used in perfumery and has not been used since 1982 when de International Fragrance Association (IFRA) first banned its use in fragrances. However, since 1995 (Api, 2006-Avalos-Peralta et al, 2005) Peru Balsam has been incorporated in fragrances as an extract or distillate, however, seems to be as allergenic as the crude product. In fact, patients allergic

to this allergen as present in the baseline series do react with the same degree of severity to the extract and distillate (Figure 8).

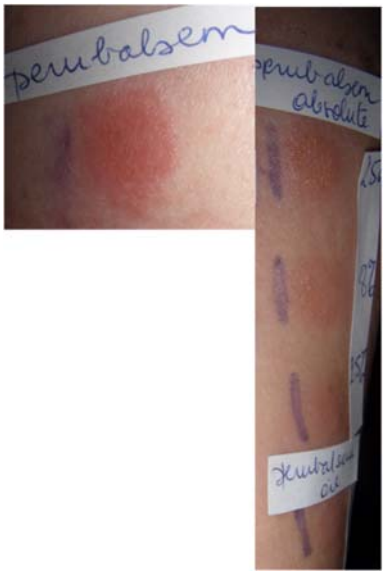

Figure 8. Strong positive patch tests to Myroxylon pereirae as tested in the baseline series (panel A) but also to the "absolute" and "oil" as used in perfumery (panel B).

Hjorth had already reported in 1961 that gums and resins such as balsam of Peru and benzoin are strong sensitizers, and associated with fragrance allergy (Hjorth, 1961) and that the most important allergens are formed by the polymerization of a 'protoresin" (monomer or low polymer), which is an ester of benzoic acid or cinnamic acid and coniferyl alcohol; however, many other allergenic components are present as well, such as benzyl cinnamate, eugenol, methyl cinnamate, benzyl benzoate, vanillin, cinnamic acid, cinnamic alcohol, cinnamal, benzyl salicylate (Larsen, 1977), all components of, or related to other allergenic fragrance materials.

2.3.3 Colophonium

Colophonium is another natural product tested in the baseline series and is obtained from pine trees, which contains allergenic oxidized resin acids (terpenes), mainly abietic and dehydroabietic acids. Simultaneous reactions are often observed with other fragrance allergens (Karlberg et al, 1988-1991), such as FM I, MP, and even compositae (asteraceae) plants, which is due to the (oxidized) terpenes present (Paulsen et al, 2005).

Of course, colophonium has mainly other applications, such as in depilating wax, glues- also for shoes, adhesives tapes, rubber, wax for musical instruments, etc. (Vandebuerie et al, 2014)

2.3.4 Fragrance Mix II

A newer screening substance has emerged from multicenter studies in order to increase the ability to diagnose fragrance allergy (Frosch et al, 2005a-b). As a result of this, since 2005, a mixture of 6 additional fragrance materials has been commercialized for introduction into the baseline series, i.e. fragrance mix II (FM II) that contains *hydroxyisohexyl 3-cyclohexene carboxaldehyde* (HICC)(Lyral®)(2,5 %), citral (1%), farnesol (2,5%), coumarin (2,5%), citronellol (0,5%), and α-hexyl-cinnamic aldehyde (5%) (FM II 14% in petrolatum). Moreover, *hydroxyisohexyl 3-cyclohexene carboxaldehyde* 5% was added to the baseline series separately as well.

HICC (*hydroxyisohexyl 3-cyclohexene carboxaldehyde***)**

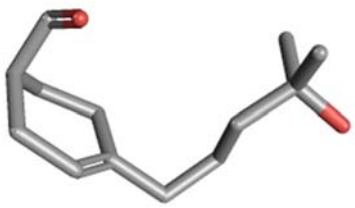

Figure 9. *Hydroxyisohexyl 3-cyclohexene carboxaldehyde* (HICC)

HICC (Figure 9) has been used for many years without restrictions. It is related to hydroxycitronellal and has probably been used as a substitute since the use of the latter had been restricted, with generally high use concentrations, being more than 3.0% in certain perfumes (Fenn, 1989). A series of systematic investigations under the leadership of Frosch have shown that HICC turned out to be one of the most frequent allergens, giving positive reactions in 1–2.7% of consecutively patch-tested patients (Frosch et al, 1995-1999-2002-2005).

2.4% of patients tested by the Danish monitoring network of dermatologists were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007); in 70% of the cases the reaction was of current relevance, i.e. causing disease (Heisterberg et al, 2010). This is in agreement with the results of a later German study, in which 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating

normal use conditions of cosmetics containing it (Schnuch et al, 2009). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing this component in realistic amounts (Jorgensen et al, 2007).

Farnesol

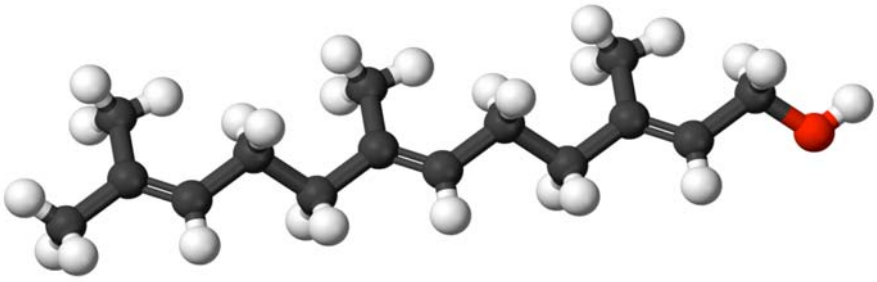

Figure 10. Farnesol

Farnesol (Figure 10) is both used as a fragrance ingredient and, at a higher concentration, also as a biocide, e.g., in deodorants (Frosch et al, 2002). It is has been shown to cause contact allergy in 1.1% of patients consecutively patch tested by the German Information Network of Departments of Dermatology (IDVK) (Schnuch et al, 2004). Those subjects positive to farnesol were characterized by being young females and having hands and face more often affected than patients negative to it (Schnuch et al, 2004). Probably, many cases of deodorant contact allergy may have been missed in the past, since most of the farnesol-positive patients are negative to FM I (Frosch et al 2002-2005-Goossens et al, 1997).

Citral

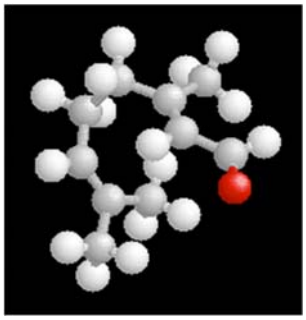

Figure 11. Citral

Citral (Figure 11) is a relatively weak allergen and has also irritant properties, which seem to be temperature dependent (Rothenborg et al, 1977). It has a steep dose–response curve (Heydorn et al, 2003a) and has been shown to be of possible significance in patients with long-term chronic hand eczema, which may be due to its combined allergenic and irritant effects (Heydorn et al 2003a-b). In European multicentre studies, 0.7–1.1% of consecutively tested eczema patients gave a positive reaction to it (Frosch et al, 2002-2005a).

Coumarin

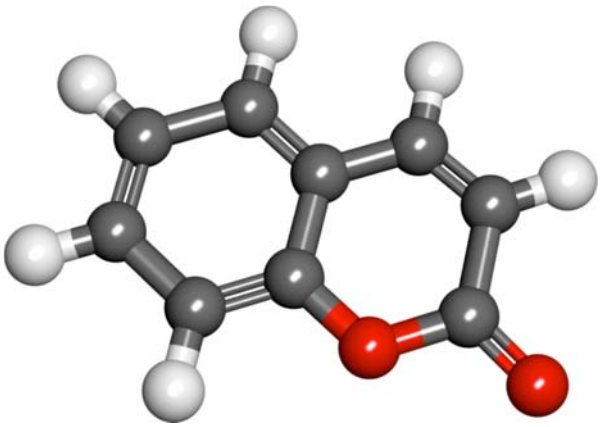

Figure 12. Coumarin

Coumarin (Figure 12) has been the subject of several studies and case investigations (Frosch et al, 2002-Mutterer et al, 1999). It has been reported to cause reactions in 0.4% of consecutively tested patients (Kunkeler et al, 1998) and also to give rise to positive reactions in 0.3% of patients in a European multicentre study (Frosch et al, 2002). However, in the most recent European investigation, it gave no reactions among 1,701 patients tested (Frosch et al, 2005a). The reason for this is unknown, but may be related to the actual use of a better quality of coumarin containing fewer sensitizing impurities.

2.3.5 Other potential fragrance-allergy markers

Limonene contained in the citrus fruits has an increasing significance, both as a solvent and fragrance ingredient, not only in fine fragrances, but also in many consumer (cosmetics, household) and industrial products, and this at rather high concentrations. The terpenes, i.e. d- and l-limonene (Skold et al, 2002-2004) easily oxidize on air exposure, and European studies have shown that

limonene (as is the case with other terpenes such as linalool and caryophyllene), are not allergenic themselves but that their oxidation products are, the strongest allergens formed being mainly hydroperoxides (Skold et al, 2002-2004). Antioxidants such as butylated hydroxytoluene (BHT) are, therefore, often added to commercial products. Testing consecutive patients in different clinics with oxidized d-limonene gave positive results in 0.3–6.5% of the cases (Matura et al, 2002). Of the patients reacting to the oxidized terpenes, 58% had fragrance related contact allergy and/or a positive history for adverse reactions to fragrance (Matura et al, 2005). Moreover, also oxidized linalool has been shown to be a frequent allergen on routine patch testing (Bråred Christensson et al, 2012).

This emphasizes the need for testing with the chemicals that are in the products and not just what was originally added, and one or more of these oxidation products certainly deserve a place in the baseline series, particularly since the patch test materials of the oxidized forms of linalool (Skold et al, 2002-2004) and limonene are now commercially available (Bråred Christensson et al, 2012, Bråred Christensson et al, 2013). In terms of prevention, expiry dates taking auto-oxidation into consideration will help to solve the problem.

Beside limonene and linalool, also linalyl acetate, alpha-terpinene and geraniol have all been identified as prehaptens. It is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product, and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitizers. Moreover, oxidized antioxidants lose their activity (Bråred Christensson et al, 2012).

Essential oils as fragrance allergens

About thousand years ago, the Arabs discovered how to extract oils from flowers by distillation and, thereby, produced essential oils. These skills spread to the Western Europe with the crusades. Essential oils come from many different plants, from a limited number of animals, and can be synthesized from two fossil oils (coal and petroleum) (EU Directive, 2003). Oils of roses, laurel and lavender are examples of essential oils obtained by steam distillation of plant raw materials, such as blossoms, leaves and fruits of flowers. Cedar-wood oil and sandalwood oil come from the wood and roots of trees (de Groot et al 1997).

Until the mid-19th century, all perfumes were blends of essential oils. Nowadays, they are still present in many perfumes, are increasingly used as bath additives, added to shampoos, in skin oils and lotions, in flower waters, or in massage oils (Scheinman, 1996), and in aromatherapy either by application to the skin, or by inhalation through vaporization. For examples, essential oils such as

ylang-ylang (YY) or sandalwood oil have gained special attention in aromatherapy and are often applied directly to the skin in high concentrations for the treatment of headache, muscular pains, arthritis, etc.

Essential oils are mostly constituted of terpenes such as α and β pinene, citral, geraniol, linalool, citronellal, hydroxycitronellal, limonene and menthol, but also contain other organic chemical compounds, including aromatics, aliphatics, alicyclics and heterocyclics. Examples of isolated substances include eugenol from cloverleaf oil, cedrol from cedarwood oil, citral from lemon grass oil, and menthol from peppermint oil. Linalool is a component of lavender, ylang-ylang, cananga, rosewood, Bulgarian rose and jasmine oils (Cockayne et al, 1997). Cinnamic alcohol is found in hyacinth oil, cinnamal in patchouli oil, eugenol in patchouli and clove oils, and isoeugenol in ylang-ylang oil. Geraniol is present in most essential oils and is the main component of rose and palmarose oil, geranium, citronella, lavender and jasmine oil (de Groot et al, 1997). The results of an European study have shown that there are at least four essential oils that have produced positive test reactions in consecutively tested patients at a frequency of over 1%, i.e. Ylang Ylang oils (I and II), lemongrass oil, narcissus absolute and jasmine absolute, which are potential additional screening agents (Frosch et al, 2002).

Chapter 3

Legislation on Fragrances

III. SAFETY MEASURES AND LEGISLATION

In order to reduce the skin hazards of fragrance materials, a good historical example of successful cooperation between dermatologists and manufacturers concerns the story of "pigmented cosmetic dermatitis in Japan", where in the 1960s, an epidemic occurred among women who presented with bizarre hyper-pigmentation of the face. Cosmetic ingredients were finally discovered as the culprit, i.e. coal-tar-derived dyes and various fragrances. On the basis of extensive patch tests studies, Nakayama and colleagues (Nakayama et al, 1984) developed the "allergen control system" for the production of safer cosmetics. Since major cosmetic companies in Japan avoided or reduced the concentration of these chemicals, the number of patients suffering from this disfiguring condition sharply declined.

The impact of sensitization to a specific allergen in the general population is mostly derived from studies in clinical patch-test populations. These findings may result into preventive and regulatory decisions, in the case of fragrance allergy -undoubtedly a matter of concern- it remains controversial as to how and against which of the different compounds preventive action should be taken (Schnuch et al, 2004). Because of the increasing importance of fragrance allergy and to ensure that sensitized consumers are adequately informed, since March 2005, 26 fragrance components have been labeled as cosmetic ingredients on the packaging (see below). Based on the expansion of our knowledge of fragrance allergy, also by results obtained within the frame of the European research project on Fragrance Allergy from April 2000 to March 2003 (EU Directive 2003), test series of fragrance substances have been updated, which enables not only to better identify fragrance-sensitive patients but also to outline the pattern of sensitivity to major and minor components. Moreover, as a result of this research project the concentration of certain fragrance chemicals, e.g. isoeugenol and hydroxy-isohexyl cyclohexene carboxaldehyde, shown to be strong allergens even in low concentrations, has been reduced.

3.1.1 The 26 fragrance allergens labeled on the packing

Ingredient labeling of 26 individual fragrance ingredients, identified as allergens in humans, was thus introduced for cosmetics in the EU in 2005: amyl cinnamal, cinnamyl alcohol, cinnamal, *Evernia prunastri* (oak moss), *Evernia furfuracea* (tree moss), eugenol, geraniol, hydroxycitronellal, iso-eugenol, alpha-isomethyl ionone, amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, citral, citronellol, coumarin, d-limonene, farnesol, hexyl cinnamal, *hydroxyl-isohexyl cyclohexene carboxaldehyde (HICC)*, butylphenyl methylpropional (lilial), linalool, methyl heptine carbonate (Annex 3 of the Cosmetic Directive). The intention was to provide a tool for clinicians for optimizing the investigation of patients with suspected fragrance allergy, as well as for fragrance allergic patients for avoiding

products containing substances to which they are allergic. Both these aims are objectives of secondary prevention and seem to have been well accepted. In a study of fragrance allergic patients and their utilization of ingredient labeling (Lysdal et al, 2009), most responded that they indeed used it (86.3%) and the majority (65.3%) of them found it helpful. Most allergic patients (83.2%) read the labels to find out if the product was scented, while 35.6% also looked for specific ingredients. Many (84.9%) considered that a clearer labeling, e.g. easier names and a larger font size, would increase their benefit.

The restrictions set are identical for all 26 substances and are detailed under "other limitations and requirements": i.e., "the presence of the substance must be indicated in the list of ingredients referred to in Article 6 (1) (g) when its concentration exceeds 0.001% in leave-on products and 0,01% in rinse-off products" (independent of their function in the products). This list of compounds was set up by the expert groups for "fragrances with possible allergenic potential" of the SCCNFP (Scientific Committee on Cosmetics and Non- Food Products) –later known as SCCP-, a selected group of mainly toxicologists working at universities and authorities advising the European Commission. The regulations on fragrances in cosmetic products were taken over for washing and cleansing products by Regulation EG/648/2004 (EC, 2004, 2006a), for which the presence of any of these 26 allergens must be indicated in the list of ingredients when its concentration exceeds 0.01%.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP previously suggested that levels of up to 200 ppm would be tolerated by the majority of sensitized individuals.

Recent voluntary restrictions (recommendations to such lower use concentrations, at least for some product types) are not reflected in available evidence. The SCCS thus considered that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, they advised that HICC should not be used in consumer products, in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized (SCCS Opinion, 2012).

3.1.2 Further restrictions and other fragrance allergens to be taken into consideration

The expert groups for fragrances with possible allergenic potential (SCCP) have been constantly evaluating the scientific literature with a systematic and critical review, in order to identify additional fragrance substances expected to be contact allergens, including natural extracts, and also fragrance substances that can act as pre- or pro-haptens, forming new or more potent allergens by air oxidation and/or metabolic activation relevant for consumers (SCCS Opinion, 2011).

Based on clinical experience, the most recent SCCP Opinion thus considered 82 substances, 54 single chemicals and 28 natural extracts (essential oils), which can be classified as established contact allergens in humans (SCCS Opinion, 2012). At this moment, following a referral to the European Commission, the SCCS has made a number of recommendations to amend Annexes II (banned substances) and III (to appear on product labeling). The ban covers HICC, atranol and chloroatranol and 12 + 8 natural extracts should be subject to mandatory labeling (ec.europa.eu, 2014).

Chapter 4

Objectives and Methods

General Objectives

1- To describe the frequency of contact allergy to fragrance allergy "markers" as tested in the baseline (standard) series, i.e. FM I, FM II, *Myroxylon pereirae* (MP) and colophonium, and to determine trends in frequency over the years. To characterize the age, sex, and lesion location of the fragrance-allergic patients and to study the association between the positive tests observed with these markers.

2- To also describe the frequency of positive reactions to the FM I- and FM II- individual components, and determine the trends in their frequency over the years. Furthermore, to determine association between positively reacting FM I ingredients and *Myroxylon pereirae* (MP), and colophonium.

3- To investigate whether the chemically modified oak moss absolute, treated by a polymer-based method and containing reduced amounts of chloroatranol and atranol, still produced positive patch-test reactions in previously sensitized subjects.

4- To identify fragrance allergens other than those tested in the baseline (standard) series.

5- To determine which and how many topical pharmaceutical products marketed in Belgium contain fragrances and to examine the nature of the fragrances in specific products that played a role in iatrogenic allergic contact dermatitis (ACD) in the patient population investigated.

6- To identify the nature of fragrance allergens responsible for ACD from specific cosmetic products.

Methods

4. Patient population

We studied consecutive patients presenting with eczematous dermatitis at the Contact Allergy Unit (Department of Dermatology) of the Katholieke Universiteit Leuven, between 1990 and 2011, and who were patch tested with a European baseline patch test series (Hermal, Reinbeck, Germany), and, when indicated, also with other contact allergens. This concerned 13,332 subjects, of whom 7486 (56%) presented with at least one positive reaction. The MOAHLFA (Schnuch et al, 1997; Uter et al, 2008) index for this patient group was: M (Male), 34%; O (Occupational dermatitis), 17%; A (Atopic dermatitis), 22%; H (Hand dermatitis), 38%; L (Leg dermatitis), 3,5%; F (Facial dermatitis), 35%; and A (Above 40 years), 48%.

The patch tests were administered with van der Bend patch-test chambers (Brielle, the Netherlands) applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Fixomull® (Beiersdorf, Germany), and later on with Mefix® (Mölnlycke Health Care, Göteborg, Sweden) as adhesive tape. The patch-test readings were performed according to the international guidelines by the ICDRG after 2 days, 3 days (exceptionally), and 4 days, and sometimes also later.

5. Data sources

The data compiled in the electronic databases developed in our center since 1978 were analyzed. Beside a patient file with clinical and patch test data, these databases also contain a product and a literature file. The product file concerns information on the complete composition of topical pharmaceutical products (n=3280) and cosmetics (n=1742) (delivered by pharmacists) in Belgium, allowing to identify the allergens present in these products. The literature file is specifically focused on contact dermatitis and includes references since 1967 (n=13.067).

6. Substances tested

The European baseline (standard) patch test series includes a 'fragrance mix', also called ‘fragrance mix I’ or FM I 8% in petrolatum, for better distinction with the newly developed 'fragrance mix II or FM II (see below). FM I is a mixture of 7 synthetic fragrances, i.e. alpha-amyl cinnamal, cinnamal, cinnamyl alcohol, eugenol, geraniol, hydroxycitronellal, isoeugenol, and the natural compound *Evernia prunastri* (oak moss absolute), all at a concentration of 1% each. The baseline series also includes *Myroxylon pereirae* [balsam of Peru (MP)] and colophonium. MP is a natural extract prepared from the exudation of the tree MP and was reported in 1961, together with other gums and resins such as benzoin, to be an important marker for fragrance allergy. The other allergenic ingredients include benzyl cinnamate, methyl cinnamate, benzyl benzoate, vanillin, cinnamic acid, benzyl salicylate, cinnamyl alcohol, cinnamal and eugenol. The latter 3 chemicals are also present in FM I.

It has been reported that as much as 33% of fragrance sensitivity may be missed if FM I is used as the only test substance to detect fragrance allergy. To compensate the unsatisfactory sensitivity of the FM I (and MP and colophonium) in the diagnosis of contact allergy to currently used fragrances, an additional marker, i.e. FM II was developed (14% in petrolatum) and introduced officially into the baseline series in 2008 (Bruze et al, 2008). FM II contains citral, citronellol, coumarin, farnesol, hexyl cinnamal, and hydroxyisohexyl cyclohexene carboxaldehyde (HICC), the

latter which is also tested separately (5% in petrolatum). Sometimes other fragrance-allergens such as the (oxidized) terpenes linalool and limonene, essential oils, and specific fragrance-containing products used by the patients have been tested as well.

Chapter 5

Frequency of and trends in fragrance allergy over a 15-year period

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Contact Dermatitis 2008: 58: 134-141

Abstract

Background: The widespread use of fragrance-containing products is probably the most important reason for its high impact in allergic contact dermatitis.

Objectives: To describe the frequency of contact allergy to fragrance allergens as tested in the standard series, in relation to age, sex and lesion locations. To determine trends in frequency over the years and to study the association between positive tests observed with the different fragrance-allergy markers as well as between specific fragrance allergens and locations of the lesions.

Patients/Methods: 10 128 patients underwent patch testing between January 1990 and December 2005 at the Dermatology department in Leuven.

Results: 1463(14.5%), that is, 380(26%)males and 1083 (74%) females, reacted positively to at least 1 fragrance-allergy marker in the standard series: 9% to fragrance mix I, 6% to *Myroxylon pereirae*, and 4.8% to colophonium (often in association), 2.1% to *hydroxyisohexyl 3-cyclohexene carboxaldehyde* and 2.1% to fragrance mix II, the latter 2 allergens having been introduced more recently. Over the years, fragrance contact allergy has shown a fluctuating trend. Hands and face were the most commonly affected body sites. Moreover, a significant association was found between specific fragrance allergens and certain locations.

Conclusions: This study illustrates that fragrance contact allergy is common in patients suffering from contact dermatitis.

Introduction

Adverse reactions to fragrances in perfumes and cosmetic products include allergic contact dermatitis (ACD), irritant contact dermatitis, photosensitivity, immediate reactions (contact urticaria), pigmented contact dermatitis, and (worsening of) respiratory problems (de Groot, 2001).

Allergic contact dermatitis from fragrances results from a combination of repeated environmental exposure and age-related susceptibility factors (Buckley *et al*, 2003) and is commonly observed in patch-test populations. Allergenic fragrance chemicals are indeed ubiquitous in our environment, not only in fine fragrances but also in a wide range of cosmetic, household, and industrial products (Matura *et al*, 2003) and even topical medicaments. Actually, together with preservative agents, they are the most important sensitizing culprits in cosmetic products (Goossens, 2006- Schnuch *et al*, 2004), with a frequency that is estimated to be around 1.5% in the general population (Denmark, 1.1% and Norway, 1.8%) (Nielsen *et al*, 1992- Dotterud *et al*, 2007) and between 6–14% in contact dermatitis patients (Marks *et al*, 1998).

The main tool for diagnosing contact allergy to fragrances is the European standard patch-test series. This standard series consists of (i) fragrance mix I (FM I), a mixture of 1 natural compound, oak moss absolute (*Evernia prunastri*), and the synthetic fragrances isoeugenol, hydroxycitronellal, cinnamal, cinnamic alcohol, eugenol, α-amyl cinnamal, and geraniol, at a concentration of 1% each, (ii) *Myroxylon pereirae* (MP or balsam of Peru), and to a less extend also (iii) colophonium. New screening substances have emerged from multi- center studies in order to increase the ability to diagnose fragrance allergy (Frosch *et al*, 2005 a-b). As a result of this, since 2005, a mixture of 6 additional fragrance materials has been commercialized for future introduction into the standard series, that is, fragrance mix II (FM II) that contains *hydroxyisohexyl 3-cyclohexene carboxaldehyde* (HICC or Lyral), citral, farnesol, coumarin, citronellol, and α-hexyl cinnamal.

The aims of this retrospective study were as follows:

(1) To describe the frequency of contact allergy to fragrance allergens as tested in the standard series.

(2) To determine trends in frequency over the years.

(3) To characterize the age, sex, and lesion location of the fragrance-allergic patients.

(4) To study the association between the positive tests observed with the different fragrance-allergy markers.

(5) To study the association between specific fragrance allergens and localization of the dermatitis.

Materials and Methods

From January 1990 until December 2005, 10 128 consecutive patients presenting with

eczematous dermatitis were patch tested in the Contact Allergy Unit of the Katholieke Universiteit Leuven with a European Standard series (Hermal, Reinbeck, Germany) and (when indicated) also with other allergens.

The patch tests were administered with Van Der Bend patch-test chambers (Van Der Bend, Brielle, the Netherlands) applied on the back with MicroporeÔ (3M Health Care, Borken, Germany) and fixed with Fixomull1 (Beiersdorf, Germany) and later on with Mefix1 (Mölnlycke Health Care, Goteborg, Sweden) as adhesive tapes. The patch-tested readings were performed according to the international guidelines by the International Contact Dermatitis Research Group (Wahlberg, 2001) after 2 days, 3 days (exceptionally) and 4 days and sometimes later.

All data were retrieved from and evaluated with a patient database developed in our department (Drieghe *et al*, 2002- Goossens, 1998). The fragrance-related allergens present in the standard series were studied in an explorative way. In the study of associations between patch- test results and the location of the lesions, the data were presented in a 2x2 contingency table. As an appropriate statistical measure to compute the strength and the direction of the association, we used the odds ratio (OR), expressing the occurrence of positive reactions in 1 group as compared with another group, and/or the relative risk, and their corresponding 95% confidence interval (CI). If the CI is different from the value 1, there is a significant association between the row and the column variables. The statistical analysis in both the male and the female populations was per- formed by using the SAS SOFTWARE SYSTEM version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Among the entire population of 10 128 patients patch tested, 3491 were males (34.5%) and 6637 were females (65.5%). 5690 (56%) presented with a contact-allergic reaction to at least 1 of the substances tested, and 1463 (14.5%), that is, 380 (26%) males and 1083 (74%) females, reacted positively to at least 1 fragrance marker, making up 26% of those with contact allergy.

The OR for females is 0.19 (odds f= 1083/ 5554 = 0.1950), expressing the probability to have a positive reaction to be 0.19 higher than the probability of having a negative reaction to fragrance. The odds for males is only 0.12 (odds m= 380/3111= 0.1221). The OR is then 1.6 with a 95% CI (1.41–1.81) or the probability of having a positive patch-test reaction in females being 1.6 times larger than in males.

The mean age in fragrance-allergic patients was 45 for males and 44 for females (both with 17 SD), compared with 39 years both for males and females (17 SD) in the non-fragrance allergic patients (n= 8665). For a more convenient handling of the data, the age of the patients was divided into groups with a span of 20 years (Fig. 1). The frequency of fragrance allergy gradually increased from young to adult age, the highest peak between 20 and 40 years (40%) for females and between

40 and 60 years (37.6%) for males, to decrease gradually again later on.

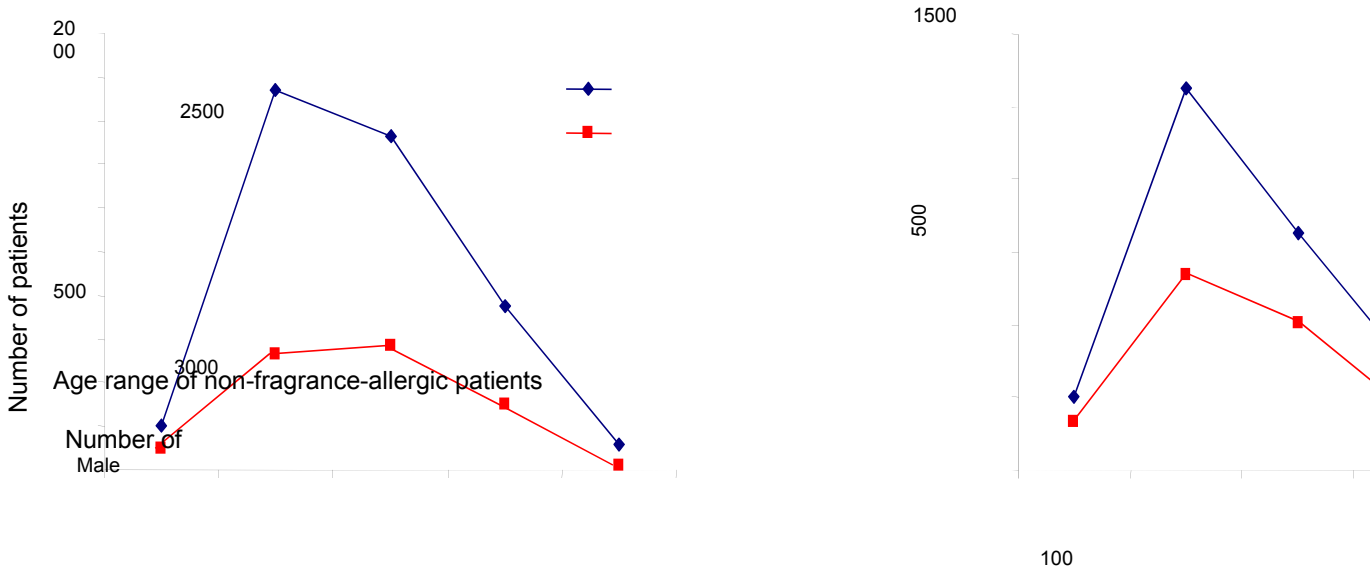

Fig. 1. Distribution of age of fragrance- (right panel) and non-fragrance allergic patients (left panel).

In the whole group, there were 3626 atopic patients (36%) (atopic dermatitis, asthma and/or allergic rhinitis), 521 of whom (14.4%) presented with a positive reaction to at least 1 fragrance-allergy marker, compared with 942 (14.5%) in the 6502 non-atopic patients (64%). Thus, positive reactions to a fragrance-allergy marker and atopy were not significantly associated.

As regarding the fragrance-allergy markers, 9% of the population tested (n= 924) presented with a positive reaction to FM I, 6% (n= 617) to MP, 4.8% (n= 489) to colophonium (Table 1), 2.1% (n= 62 in 2901 patients tested) to HICC, and 2.1% (n= 7 in 335 patients tested) to FM II, the latter allergens being introduced more recently, in 2002 and 2005, respectively. 30 patients out of 62 (48.4%) reacted both to FM I and to HICC. The total number of positive reactions observed is, of course, higher than the number of patients as many of them suffered from multiple sensitivities. Among those reacting to FM I, MP, or colophonium, females represented 72%, 74%, and 77% and the males 28%, 26%, and 23%, respectively.

Table 1. Positive reactions observed to fragrance mix I, *Myroxylon pereirae* and colophonium

Markers	Total (*n*= 10.128)	%	Male (*n*= 1463)	Female (*n*= 6637)
FM I	924	9.12	258	666

MP	617	6.09	162	455
C	489	4.83	113	376

FM I, fragrance Mix I; MP, *Myroxylon Pereirae*; C, colophonium.

The results of the positive patch-test reactions obtained with FM I, MP, and colophonium over the years are given in Table 2, which is illustrated in Fig. 2.

Table 2. Percentage of positive patch-test results obtained with fragrance mix I, *Myroxylon pereirae* and colophonium over the years

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
FMI	7.2	7.3	6.6	5.6	13.1	9.6	10.1	8.1	11.9	13.9	12.5	9.7	8.4	8.3	9.3	7.7
MP	6.5	6.8	5.2	4.8	4.8	6.5	6.7	5.6	6.1	6.8	7.7	6.9	6.6	6.3	7.9	5.0
C	5.5	5.5	3.7	3.8	6.1	4.8	4.7	3.7	6.3	5.7	5.6	5.4	4.6	4.3	5.3	4.3

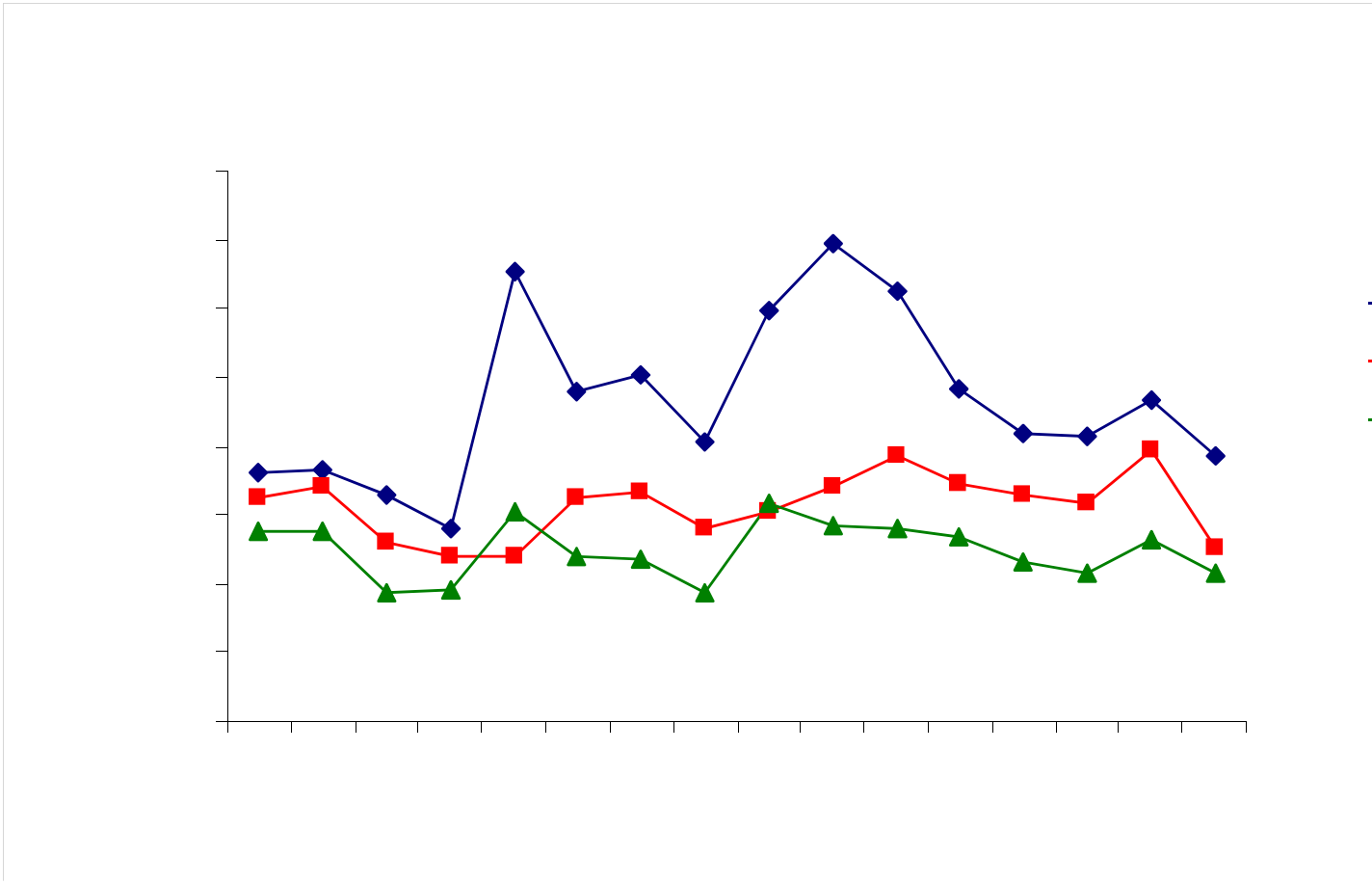

Fig. 2. Evolution of the relative frequency over the years of positive reactions to fragrance-allergy markers. C, Colophonium; FM, Fragrance Mix I; MP, *Myroxylon Pereirae*.

The associated positive tests observed with the different fragrance-allergy markers are given in Table 3 (multiple hypotheses testing for all the different fragrance-allergy markers were taken into account), showing a strong association for all comparisons reported. When the associations were significant, the OR was computed. As an example, an OR of 11 (Table 3) means that the odds of a positive reaction to colophonium is 11 times higher for patients with a positive reaction to FM I than for non-FM I allergic patients.

Table 3. Odds ratios and corresponding 95 % CIs for the different fragrance-allergy markers

	Males		Females	
Allergens	OR	(95 % CI)	OR	(95 % CI)
FM I and C	11.14	(7.48-16.59)	5.11	(4.05-6.46)
FM I and MP	35.74	(24.98-51.15)	18.96	(15.35-23.42)

FM I and HICC	25.63	(6.37-103.10)	17.50	(10.09-30.34)
C and MP	5.95	(3.65-9.70)	5.35	(4.14-6.92)

C, Colophonium; CI, confidence interval; FM, fragrance mix I; HICC, *hydroxy-isohexyl cyclohexene carboxaldehyde*; MP, *Myroxylon Pereirae*; OR, odds ratio.

The localization of the lesions in the fragrance (n=1463) and non-fragrance (n= 8665) allergic patients are visualized in Fig. 3. Hands (31.2%), face (27.2%), lower legs (16.6%), and feet (15.3%) were the most commonly affected body sites infragrance-sensitive individuals, particularly in females. Significant associations were found for arms (relative risk, 1.24; 95% CI, 1.09–1.41), lower legs (relative risk, 1.71; 95% CI, 1.51–1.93) and upper legs (relative risk, 10.82; 95% CI, 8.56– 13.68), and axillae (relative risk, 1.51; 95% CI, 1.21–1.90). For hands (relative risk, 0.8; 95% CI 0.76–0.93), there is a negative correlation with fragrance allergy though.

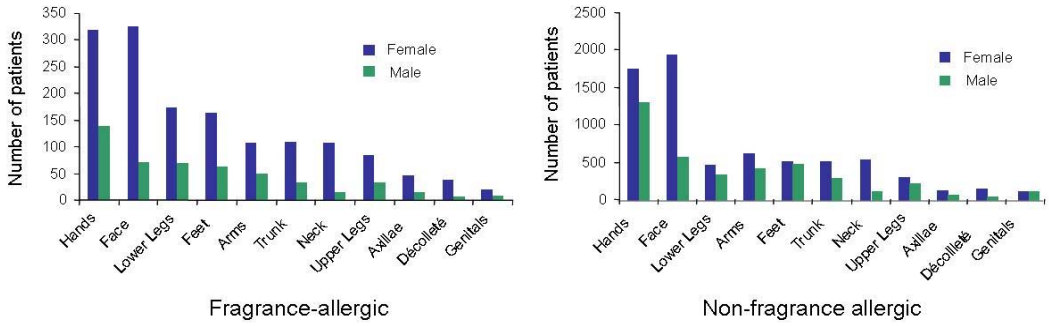

Fig. 3. Visualisation of localisation of the lesions in fragrance-allergic (*n*= 1463) (right panel) and non-fragrance allergic patients (*n*= 8665) (left panel).

Table 4 shows the results of the association between localization and specific fragrance allergens. Of 40 combinations of localization by marker, 9 were significantly associated either for males or for females, or both. As this was an explorative study, a correction for multiple comparisons was not used.

Table 4. Odds ratios and corresponding 95 % CI for the association between localizations and the fragrance allergens

	Males		Females	
Allergens	OR	95 % CI	OR	95 % CI

Axillae and FM I	2.32	(1.29-4.16)	2.22	(1.54-3.20)
Axillae and HICC	4.65[ns]	(0.57-37.63)[ns]	7.28	(3.62-14.62)
Trunk and HICC	1.20[ns]	(0.15-9.64) [ns]	2.02	(1.01-4.02)
Upper-leg and C	1.17[ns]	(0,60-2.26)[ns]	1.48	(1.01-2.16)
Upper-leg and MP	1.23 [ns]	(0.71-2.13) [ns]	1.50	(1.06-2.12)
Lower-leg and FM I	2.17	(1.58-2.99)	1.94	(1.55-2.43)
Lower-leg and MP	2.07	(1.39-3.06)	2.20	(1.71-2.84)
Lower-leg and C	1.96	(1.22-3.13)	2.10	(1.59-2.77)
Feet and C	2.14	(1.40-3.25)	2.62	(2.03-3.39)

C, Colophonium; CI, confidence interval; FM, fragrance mix I; HICC, *hydroxy-isohexyl cyclohexene carboxaldehyde*; MP, *Myroxylon Pereirae*; NS, non significant values; OR, odds ratio.

Discussion

It is well known that after nickel, contact allergy to fragrance components is the most common finding among contact-allergy patients (de Groot *et al*, 1997-Nielsen *et al*, 1993). This was also true in our female population studied and, since 1995 to 2006, FM I and p-phenylenediamine (data not shown) were, in alter- nation, the 2 most frequent allergens in our male population. The majority of patients with fragrance sensitivity were women (Scheinman, 1996-Malanin *et al*, 1989-Malten *et al*, 1984), which reflects a greater exposure to fragranced cosmetics in this population. However, in view of an increasing usage also in men, the sex difference may become less prominent in the future.

Buckley et al. argued that ACD from fragrances has an age-related susceptibility (Buckley *et al*, 2003) and observed a peak in the 60s for females and in the 70s for males (Buckley *et al*, 2003). The highest peak observed between the ages 20 and 40 in our female population could be explained by the greater personal use of fragranced products in young women in particular. In males, the highest peak was observed between the ages 40 and 60. This could perhaps be explained by a different patient selection or a different consumer exposure in both centres.

With regard to the fragrance-allergy markers, FM I has been regarded as an adequate screening test for fragrance sensitivity, although it has been reported that as much as 33% of fragrance sensitivity may be missed (Larsen *et al*, 1998), if it is used as the only test substance. This percentage is probably too low and depends on which fragrance chemicals or products the subjects are tested with. Indeed, the more fragrance allergens tested, the more positive reactions can be observed. In most epidemiological studies, as well in the current study, the patch-test result of

patients reacting to their own perfume and deodorant, for example, or to any other fragrance allergen, have not been considered. Besides FM I, our results also take into consideration MP and colophonium, for which, in agreement with other studies, including a recent report from Austria (Wohrl *et al*, 2001), positive reactions are significantly associated in both sexes. Indeed, this can be explained by the fact that these products share similar components.

In fact, Hjorth has already reported in 1961 that gums and resins such as MP and benzoin are strong sensitizers, associated with fragrance allergy (Hjorth, 1961). He showed that the most important allergen of MP is formed by the polymerization of a 'protoresin' (monomer or low polymer), which is an ester of benzoic acid or cinnamic acid and coniferyl alcohol; however, many other allergenic components are present such as benzyl cinnamate, eugenol, methyl cinnamate, benzyl benzoate, vanillin, cinnamic acid, cinnamic alcohol, cinnamal, and benzyl salicylate (Larsen, 1977), all present in or related to other allergenic fragrance materials. The crude MP, as such, has not been used in perfumery since 1982, when the Inter- national Fragrance Association banned its use in fragrances. Since 1995 (Api, 2006a- Avalos-Peralta *et al*, 2005), MP has been incorporated in fragrances as an extract or distil- late, but could be, according to a recent study, as allergenic as the crude product (M. Bruze, Malmo "University Hospital, Lund University, Malmo", Sweden, personal communication).

Colophonium, originating from pine trees (Karlberg *et al*, 1988), often co-reacts with fragrance allergens; their major constituents are resin acids, that is, abietic acid and dehydroabietic acid, of which the oxidized derivatives are responsible for contact allergy (Karlberg, 1991). The same allergens have also been identified in the FM I component '*Evernia prunastri*' or 'oak moss', either as contaminants or as ingredients of '*Evernia furfuracea*' or 'tree moss', also derived from pine trees and often used by the perfume industry as a cheaper substitute for oak moss (Johansen *et al*, 2002-Lepoittevin *et al*, 2000). Besides oxidized terpenes, however, oak moss contains a large number of other ingredients, including atranalol and chloroatranalol, which are perhaps among the most allergenic substances ever identified (Johansen *et al*, 2002), that is, still a 0.1 p.p.m. (the individual components of FM I will be discussed separately in a further study).

Besides FM I, MP, and colophonium, positive reactions to FM II, in which HICC is the most common sensitizer (Frosch *et al*, 2005 a-b), and HICC itself were also considered, although tested in a minority of the patients. The percentage of positive reactions found for HICC (2.1%) is in agreement with the prevalence found in other European studies, that is, 1.5–3% (Frosch *et al*, 2005 a- Frosch *et al*, 1999-Frosch *et al*, 2002-Baxter *et al*, 2003-Nardelli *et al*, 2004). This is in contrast to North America where the prevalence was found to be only 0.4% (Belsito *et al*, 2006), the difference being attributed to the presence of HICC in high concentrations in deodorants in the EU, likely to induce sensitization in occluded areas. Contact allergy to a fragrance would have been

missed in 32 out of 62 (52%) patients if we had only tested with FM I and not with HICC. This is much higher than what has been reported by Frosch et al., that is, in 7.2% of the patients (Frosch *et al*, 1999-2002). Because of the low number of patient tested and reacting to FM II, we are not able to formulate any conclusion about missed reactions to this mixture. Frosch et al. previously reported that 1/3 of the patients reacting to FM II were negative to FM I (Frosch *et al*, 2005 b). Geier et al. in a large German study reported that 535 out of 6968 (7.7%) patients reacted to FM I, while 321 of 6968 (4.6%) were positive to FM II. 151 of them reacted to both allergens, representing 28% of those with contact allergy to FM I and 47% of those allergic to FM II. FM II has thus acceptable diagnostic qualities and is helpful as an additional tool in detecting fragrance allergy (Geier *et al*, 2006).

Concerning the trends in the fragrance sensitivity rate (Table 2 and Fig. 2), a fluctuating trend, either increasing or decreasing, was observed. For FM I, the frequency was highest from 1998 to 2000 and has then decreased in recent years. In 2006 (data not shown), the frequency was the same as in 2001, that is, 9.65. In the past decade, the prevalence of fragrance sensitivity, evaluated by testing only with FM I, varied considerably. A percentage between 7 and 10 was observed among patch-tested patients in Europe (Schnuch *et al*, 2004- 1997-Temesvari *et al*, 2002-Bangha *et al*, 1996-Hasan *et al*, 2005- Buckley *et al*, 2000). In Denmark, it rose from 4.1% in 1985–1986 to 9.9% in 1997–1998 (Johansen *et al*, 2000), and a similar rising tendency was reported in Slovenia as well (Lunder *et al*, 2000). However, a recent decrease in FM I sensitivity has been reported in several studies (Schnuch *et al*, 2004- Larsen *et al*, 1998- Wohrl *et al*, 2001- Schnuch *et al*, 1997- Temesvari *et al*, 2002), for example, according to a recent European multi-centre survey, the fragrance sensitivity rate has decreased significantly from 13.1% in 1999 to 7.8% in 2002 (Bruynzeel *et al*, 2005). This was also observed in the USA, where the fragrance sensitivity rate diminished from 14% to 11.4% in the late 1990s (Marks *et al*, 1998-Marks *et al*, 2000) and, according to a more recent study, even down to 5.9% (Belsito *et al*, 2006). In the future, perhaps a further decrease in positive reactions to FM I allergens may be expected. Indeed, because of the labelling of 26 fragrance components by EU legislation (EU Directive, 2003), including those of FM I, as well as the outcome of recent dermatological studies on fragrance allergy and subsequent legal requirements [for example, reducing the concentration of isoeugenol (Tanaka *et al*, 2004)], the policy of some fragrance companies has changed. For instance, it has been shown that the new prestige perfumes contain less often or lesser amounts of fragrance chemicals included in FM I, compared with the older perfumes (Rastogi *et al*, 2003). Moreover, deodorants and domestic and occupational products quite often contain fragrance chemicals different from those present in FM I, such as limonene (Rastogi *et al*, 1998- Rastogi *et al*, 2001). This underlines the usefulness of new screening substances for the purpose of increasing the ability to diagnose fragrance allergy (Frosch

et al, 2005 a-b). In 2005, Hasan et al. have shown that in contrast to FM I, there is a significant increase in the sensitivity rate to MP in recent years (Hasan *et al*, 2005), whereas the sensitivity rate to MP has been rather stable over the years in our study.

As to the lesion localization, according to our study, hands, face, lower legs, and feet are the most commonly affected body sites. Although fragrance allergy is not significantly associated with hand dermatitis, the hands are the most frequently involved sites in fragrance-allergic women (in men, hand dermatitis is more often because of other, more occupation-related allergens). This is not surprising (Johansen *et al*, 1996-Heydorn *et al*, 2003 a-b) as many contactants do indeed contain fragrances: household products, cleaners, waxes, polishes, textile softeners, etc., and, of course, nearly all cosmetics (and many topical medications) are applied by the hands, sometimes also on the skin of other family members or pets (Scheinman, 2002). Moreover, patients may suffer first from irritant or atopic hand dermatitis, which may later be complicated by contact allergy to products used for treatment (fragranced topical drugs) or prevention (hand creams and lotions) or to other contacted perfumed products. Last but not least, dyshidrotic eruptions have been ascribed to ingestion of spices (Meynadier *et al*, 1986), which may contain fragrance chemicals or may cross-react with them (Lehucher-Michel *et al*, 2000).

As to the face, the sensitive facial skin and the eyelids in particular are susceptible to develop ACD from fragranced skin-care products, decorative cosmetics, and cleansing preparations and sometimes even from airborne exposure (Dooms-Goossens, 1993a-Dooms-Goossens, 1993b). Moreover, microtraumata from shaving facilitate (photo) contact allergy to aftershave fragrances (Edman, 1994), but also other skin diseases such as atopic dermatitis and even facial psoriasis may be induced or aggravated by ACD from fragrances (de Groot *et al*, 1983). In our population, although the face was a common localization, the association with fragrance allergy was not significant. Indeed, preservative agents, in particular, and, to a lower extend, emulsifiers are also important cosmetic allergens causing ACD on the face.

Significant associations between the neck, skin behind the ear or de´ collete´ that are other usual sites of fragrance contact dermatitis were not observed. In these areas, nickel and cobalt present in cheap metal jewels are common allergens as well. The latter body sites as well as the axillae, notwithstanding its significant association with 'fragrance' allergy (Table 4), are certainly under- represented in our study. Indeed, in general, the relation with the causal product being obvious (Goossens *et al*, 1997- Svedman *et al*, 2003), patients consult because of dermatitis located elsewhere (which is indicated in our patient file as being the first site affected), and, after identification of fragrance allergy, they then often recall having previously suffered from skin reactions to toilet waters or deodorants, respectively. Reactions to FM I and to HICC (in females) were significantly associated with locations in the axillae, as has been reported earlier (Johansen *et*

al, 2003).

Contact allergy to FM I, colophonium, and also MP is often found in patients, especially females suffering from leg ulcers, who are exposed to fragrance-containing medications, adhesive tapes, etc. This will be investigated separately in a further study on iatrogenic dermatitis caused by fragrances. As to the feet, colophonium is certainly the main culprit in shoe dermatitis, not as a fragrance allergen, of course, but because it is used as a tackifier (Nardelli *et al*, 2004).

Significant, but perhaps unexpected associations between fragrance allergy and locations such as the trunk, arms, and also upper legs were found in females, although not with a specific fragrance chemical. This most probably reflects the use of fragrance-containing body creams and lotions and perhaps skin-cleansing products, in which, besides preservative agents, fragrances are the most important allergens. Furthermore, other existing eczematous conditions such as those located in the perianal or vulvar area (Nardelli *et al*, 2005) may also be complicated by fragrance allergy.

Conclusions

This study illustrates that also in our department, fragrance contact allergy is commonly observed in patients suffering from contact dermatitis, both in males and in females, and has shown a fluctuating trend over the years. Positive patch tests to the fragrance-allergy markers, that is, FM, *M. pereirae* (balsam of Peru), and colophonium in the standard series are frequently associated. The hands and face were the most commonly affected body sites, and significant associations were found for legs, arms, and axillae. Some fragrance allergens are specifically related to certain lesion localizations. Moreover, although HICC and FM II were only more recently introduced in the standard series, a significant association was found between HICC and axillae (in females).

However, not only the fragrance-allergy markers present or those being introduced in the standard series need to be taken into account, but also the products used by the patients, essential oils and other individual fragrance allergens as this certainly increases the detection of fragrance allergy (data to be published).

Special attention to safety assessments must be made for fragranced products and, particularly, for those intended for use on potentially traumatized skin, that is, hands, beard region, shaved legs, occluded areas or areas of high absorption, that is, eyelids, axillae, and genitals, or in chronic dermatitis, that is, stasis dermatitis/leg ulcer patients.

Acknowledgement

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Chapter 6

Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals?

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Abstract

Background: Chloroatranol and atranol are degradation products of chloroatranorin and atranorin, respectively, and have been identified as important contact allergens in oak moss absolute (*Evernia prunastri*).

Objectives: To investigate whether chemically modified extracts of oak moss produce positive patch test reactions in previously sensitized subjects.

Methods: A sample of oak moss was treated by a polymer-based method to reduce the content of the two main allergens, namely atranol and chloroatranol, from 3.4% to less than 75 p.p.m. and from 1.8% to less than 25 p.p.m., respectively. Fourteen subjects with positive reactions to oak moss from Trolab and/or Chemotechnique were patch tested to this sample, diluted 1% in petrolatum.

Results: The chemically modified sample reacted negatively in six but gave still positive reactions in eight subjects, with the same intensity as the commercially available oak moss patch test materials.

Conclusions: Polymer based treatment of oakmoss extract reduces the allergenic elicitation potential in previously sensitized individuals only to a minor extent. The residual concentrations of atranol and chloroatranol being less than 75 p.p.m. and 25 p.p.m., respectively, are unsafe for the consumer.

Oak moss absolute, a popular natural extract derived from the lichen Evernia prunastri, is widely used in perfumery because of its woody aroma and fixative properties (Larsen *et al*, 1998; Actander, 1960). It has been identified as a major cause of allergic reactions, accounting for at least 20% of the reactions observed with the Fragrance Mix I (Larsen *et al*, 1998;Buckley *et al*, 2000; Schnuch *et al*, 2004). For this reason, oak moss absolute is part of the diagnostic screening test for fragrance contact allergy (Larsen *et al*, 1998). Chloroatranol and atranol have been identified as the main allergens in oak moss absolute by a bioassay-guided chemical fractionation procedure. Both substances are not found to be significantly present in the natural form of oak moss. However, during the production of the fragrance material, they can be formed by decomposition of depsides present in the lichen such as chloroatranorin and atranorin, which are non-volatile and odourless substances (Bernard *et al*, 2003). The content of atranol and chloroatranol in cosmetic products depends upon the oak moss absolute used, upon the degradation of atranorin and chloroatranorin in the product matrix, and, finally, upon the matrix effect in the determination of these molecules (Rastogi *et al*, 2004).

It has been found that oak moss absolute contains approximately 2.1% (w/w) of atranol and 0.9% (w/w) of chloroatranol (Bernard *et al*, 2003). For this reason, exposure seems to be greater to atranol than to chloroatranol in perfumes. Indeed, quantitative exposure to chloroatranol and atranol has been studied in some popular perfumes, eaux de parfum and eaux de toilette available on the European market. Thirty-one products were analysed by liquid chromatography–electrospray ionization tandem mass spectrometry (LC- ESI-MS-MS) for their contents of atranol and chloroatranol. The two substances were found in 87% (n = 27) of the products investigated, atranol in a median concentration in perfumes of 0.5 p.p.m. and chloroatranol of 0.25 p.p.m., with a maximum of 190 p.p.m. and 53 p.p.m., respectively (Rastogi *et al* 2004; Johansen *et al*, 2006). In 2007, Rastogi et al. (Rastogi *et al*, 2004; Rastogi *et al*, 2007) observed a significant decrease in the frequency of presence of chloroatranol in the products analysed in comparison with the above-mentioned study.

The sensitizing capacity of chloroatranol and atranol, as well as of oak moss absolute, has been studied in the local lymph node assay, and all proved to be strong sensitizers. It was shown that less chloroatranol was required than atranol to induce sensitization (SCCP, 2004). Moreover, it was also shown that chloroatranol was more likely to pro- duce a patch test response than atranol when tested in equimolar concentrations in sensitized individuals. The elicitation capacity of chloroatranol relative to atranol was 217%, i.e. a factor of 2.2 based on molar concentrations (Johansen *et al*, 2006). The difference in exposure to atranol and chloroatranol may, therefore, counterbalance the difference in elicitation capacity with regard to clinical importance.

The Scientific Committee on Consumer Products (SCCP), an independent advisory committee

to the European Commission, recommended in 2004 that chloroatranol and atranol should not be present in any cosmetic product because of their potency as allergens and considered untreated oak moss to be unsafe for the consumer (SCCP, 2004). Therefore, an important challenge today for the cosmetic industry is to remove selectively the oak moss absolute allergy-causing components in a fashion that will not affect most of the other components of the extract neither its olfactive properties.

To improve the skin tolerance to atranol and choroatranol, Ehret et al. (Ehret *et al*, 1992) described the treatment of oak moss absolute with amino acids such as lysine and leucine, which lowered the content of allergenic constituents. The resulting oak moss absolute was tested in comparative studies on guinea-pigs and healthy volunteers. The results of the guinea-pig maximization test and human repeated insult patch test indicated that, in comparison with the commercial test sample, the allergenicity of the new oak moss absolute was considerably reduced. However, appropriate clinical studies on individuals with characterized contact allergy to oak moss have never been performed, to our knowledge, and are still required to demonstrate the dose–response characteristics of elicitation reactions with oak moss-treated preparations.

An elegant and efficient procedure reported in the literature for the removal of allergenic components of some essential oils was based on their binding to an insoluble polymer support through a mild coupling reaction (Cheminat *et al*, 1980, Cheminat, 1981). As an example, the use of aminoethyl-polystyrene in ethanol was described to allow the removal of allergenic α- methylene-γ-butyrolactones from costus oil by Michael addition of the polymer nucleophilic ends with the electrophilic conjugated system of the lactones. In the course of our studies on allergenic natural extracts, we adapted this methodology to oak moss in order to remove sensitizing molecules such as aldehydes atranol and chloroatranol. The current clinical study was undertaken to investigate whether this chemically modified oak moss absolute, treated by a polymer-based method and containing less amounts of chloroatranol and atranol, still produced positive patch test reactions in previously sensitized subjects.

Materials and Methods

A sample of oak moss absolute was treated by a polymer-based method to reduce the content in atranol and chloroatranol, the two main allergens present in oak moss, at the Laboratory of Dermatochemistry, University Louis Pasteur, Strasbourg, France. The natural extract was trea- ted with a polystyrene derivative having a specific reactivity towards aldehydic chemical functions. After treatment, the polymer containing the alde- hydes bound was filtered-off, the solvent of the filtrate was removed under reduced pressure, and the remaining product was the polymer-treated oak moss absolute (sample A). It was possible to recover afterwards the polymer-bound aldehydes

through chemical treatment. Atranol and chloroatranol contents were decreased (sample A) from 3.4% to less than 75 p.p.m. and from 1.8% to less than 25 p.p.m., respectively (high- performance liquid chromatography–ultraviolet measurements).

From February 2004 until November 2006, 14 patients, 4 males and 10 females (age range 24–76 years), known to be sensitized to oak moss absolute, were investigated at the Contact Allergy Unit in Leuven. They were retested to oak moss absolute from Trolab and Chemotechnique and patch tested to sample A, diluted in a 1% concentration in petrolatum. The patches were applied to the upper back using either Finn Chamber® with filter discs affixed with Scanpor® tape or Van Der Bend patch test chambers (Van Der Bend, Brielle, the Netherlands) on Micropore ™ (3M Health Care, Borken, Germany) and fixed with Mefix1 (Mölnlycke Health Care, Göteborg, Sweden).

The patch tested readings were performed according to the international guidelines by the International Contact Dermatitis Research Group (Wahlberg, 2001) after 2 days and 4 days, except in six subjects who did not want to come back for the second reading, but who were asked to report on additional late positive reactions by telephone.

Results

Table 1 summarizes the patch test results. All 14 subjects had a positive reaction to oak moss absolute, from Trolab, Chemotechnique, or both. In six of them, negative reactions to sample A were observed: three had negative readings at D2 and D4 and the other three reacted negatively at D2 and did not report on additional late positives. Eight subjects reacted to sample A with the same intensity as the commercially available oak moss patch test materials.

Table 1. Patch test results at day 2 and day 4

Patient	Oak moss Trolab		Oak moss Chemotechnique		Oak moss Sample A		Other
	D2	D4	D2	D4	D2	D4	
1	+	NR	_	NR	+	NR*	FM I
2	_	_	_	+	_	NR*	FM I
3	+++	NR	+++	NR	_	NR*	FM I
4	+?	NR	+	NR	+	NR	FM I, colophonium
5	+	NR	+	NR	+	NR	FM I
6	+	NR	+	NR	_	NR*	FM I, colophonium

7	+?	+?	+	+	_	_	FM I
8	+	+	+	_	+?	+	FM I
9	+	++	+	++	+	++	FM I
10	+	++	_	_	+?	++	FM I
11	+?	+	+?	+	+?	+	FM I
12	+	+	_	+?	_	_	FM I
13	_	+	_	_	_	+?	FM I, colophonium
14	_	++	_	++	_	_	FM I

+?, doubtful reactions; FM I, fragrance mix I; NR, not read.

* Did not report on additional late positive reactions.

Figures 1 and 2 illustrate the positive patch test reactions observed in patient number 3. In Fig. 2, extreme positive reactions with intense erythema, infiltration, and coalescing vesicles to oak moss absolute from Trolab and Chemotechnique are visible, whereas the treated oak moss sample A is negative. Additionally, the patient also reacted to other components of Fragrance Mix I, i.e. isoeugenol and eugenol.

Fig. 1. Positive patch-test reaction to Fragrance Mix I (subject 3).

Fig. 2. Positive patch-test reactions to the ingredients in the same patient, the chemically modified sample A (0M010) being negative.

Discussion

According to the EU Cosmetics Directive, the maximum authorized concentration of oak moss absolute in cosmetic products is 0.1% (Council Directive/EEC, 1976). An oak moss sample was found to contain approximately 2.1% atranol and 0.9% chloroatranol (Bernard *et al*, 2003). Considering this as representative of atranol and chloroatranol in different oak moss absolutes samples, cosmetic products may contain up to 0.0021% (21 p.p.m.) atranol and 0.0009% (9 p.p.m.) chloroatranol. With regard to elicitation, chloroatranol was shown to cause reactions at the p.p.m. level (0.0005%, i.e. 5 p.p.m.) by repeated open exposure and at the p.p.b. level on patch testing (50% reacted to an extreme low concentration at 0.000015%, i.e. 150 p.p.b.) (Johansen *et al*, 2003). Judged from this elicitation profile, chloroatranol is considered to be the most potent allergen present in consumer products today.

In 2008, based on new experimental sensitization data on chemically treated and untreated oak moss absolute samples, the SCCP was of the opinion that treatment of oak moss absolute at a lab- oratory scale was able to reduce the levels of atranol and chloroatranol to less than 2 p.p.m. each. Therefore, the levels of these allergens in cosmetic products in which oak moss is used at 0.1% would be such that the risk of induction and elicitation of allergic reactions to them would be low. However, the SCCP still considers that any reduction in elicitation will need to be

demonstrated by appropriate clinical testing of subjects previously sensitized (SCCP, 2004).

In the clinical study presented here, a chemically modified oak moss absolute, by using a polystyrene derivative with specific reactivity towards aldehydic chemical functions, was used to investigate if it still produced positive patch test reactions in previously sensitized subjects. The chemical treatment with the polymer gave a quality of oak moss with a low content of atranol and chloroatranol. It was estimated that atranol and chloroatranol contents were decreased from 3.4% to less than 75 p.p.m. and from 1.8% to less than 25 p.p.m., respectively. The patch test results presented here showed that treated oak moss produced elicitation reactions in 8 of 14 oak moss previously sensitized subjects. Therefore, it must be considered that the residual concentrations of atranol and chloroatranol being less than 75 p.p.m. and 25 p.p.m., respectively, are still far too high to be safe regarding elicitation.

However, we did show in a previous study that methyl-β-orcinol carboxylate, a depside degradation product and the most important monoaryl derivative of oak moss from an olfactory stand point, was also eliciting a reaction in patients sensitized to oak moss included in the study, although to a lower extent compared with atranol and chloroatranol (Bernard *et al*, 2003). In reality, freshly harvested oak moss has no scent. The moss contains various types of depsides, which are non-volatile and odourless such as atranorin and chloroatranorin (Ter Heide *et al*, 1975). The characteristic oak moss fragrance is developed after cleavage of the depsides under treatment of the oak moss concrete with alcohols to give volatile, scented monoaryl derivatives (Boelens *et al*, 1993). As such, the transesterification and decarboxylation of atranorin and chloroatranorin during the ethanolic treatment of the moss gives not only atranol and chloroatranol but also methyl-β-orcinol carboxylate, which is the major responsible for the characteristic earthy like odour of oak moss products (Bernard *et al*, 2003). The treated oak moss absolute used in this study was obtained after a polymer-based treatment that was only specific for the removal of aldehyde derivatives such as atranol and chloroatranol. Therefore, methyl-β-orcinol carboxylate was still present in the tested sample A. As this compound was shown to elicit allergy reactions to previously sensitized oak moss patients and as it was not removed by the chemical treatment of the moss, it might be also suggested that the methodology used for that treatment did not completely eliminate the eliciting potential of oak moss absolute, so could also be an extra factor to explain why some patients still elicited positive reactions to the treated oak moss.

Conclusions

We could show that polymer-based treatment of oak moss extract reduces the allergenic elicitation potential in previously sensitized individuals only to a minor extent. In view of more recent data

and considering elicitation, the major sensitizers atranol and chloroatranol should be eliminated almost completely from oak moss absolute in order to be safe for consumers.

Chapter 7

Allergic contact dermatitis from fragrance components in specific topical pharmaceutical products in Belgium

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Abstract

Objectives: To determine which topical pharmaceutical products marketed in Belgium contain fragrances and to examine the nature of the fragrance allergens in specific pharmaceutical products having caused iatrogenic contact dermatitis.

Methods: All topical pharmaceutical products marketed in Belgium, that is 3820 products, were examined as to their fragrance content as labelled. Data of 18 960 patients investigated for contact allergy between 1978 and 2008 were retrieved from our database, including information on the nature of the topical pharmaceutical products used, the results of patch tests, and the sensitization sources.

Results: Three hundred and seventy (10%) of 3280 of the topical pharmaceutical products were found to contain a total of 66 fragrance substances. Among 3378 patients suffering from iatrogenic allergic contact dermatitis, 127 were found to react to 48 specific products, for which 38 different fragrance substances gave relevant positive reactions. Women were more affected than men, and legs, hands, and face were the most commonly affected body sites.

Conclusions: Fragrances, the presence of which is in most cases unnecessary, do contribute to iatrogenic allergic contact dermatitis. Moreover, sensitized patients have difficulties in avoiding their specific allergens because standardized labelling of the ingredients in pharmaceutical products is lacking.

Contact allergy to fragrances is common, the frequency of which in the general population is estimated to be around 1.5% (Denmark, 1.1% and Norway, 1.8%) (Nielsen *et al*, 1992; Dotterud *et al*, 2007; White *et al*, 2006) and between 6.5% and 10.4% in contact dermatitis patients (Frosch *et al*, 2005a; Pratt *et al*, 2004). The real incidence of adverse reactions to topical medicaments is not known, and most of the data about prevalence are quite old with a frequency estimated to be between 14% and 18% among patients investigated for contact allergy (Brandão *et al*, 2006). In our contact allergy unit, topical pharmaceutical products account for about 33% of all contact allergies, the allergenic culprits being active principles, preservatives, vehicles, and also fragrance components (Brandão *et al* 2006, Brandão *et al*, 2001).

Labelling of all pharmaceutical products has been required in Belgium since the end of the 19th century, that is active principles and also inactive ingredients (Dooms-Goossens *et al*, 1980). Regarding the fragrances, their precise nature or a statement such as 'perfume' can be found, either on the packaging or on the notice (leaflet). In Europe, such labelling has been required since 1965, and only the active ingredients needed to be taken into account (De Greef *et al*, 1978). The list of ingredients to be labelled in 1965 was, however, revised in 2001: additional particulars needed to be stated on the outer packaging of medicinal products, such as the list of all excipients in the case of injectable products or of topical or eye preparations (EU Directive, 2001). However, there is no restriction as to the presence of fragrance allergens nor are the ingredient names standardized.

The fragrances present may be natural products, such as essential oils (e.g. orange flower oil and lavender oil), resins [e.g. benzoin and balsam of Peru (*Myroxylon pereirae*)], semisynthetic chemicals (produced from isolates through chemical reactions, such as terpineol from pinene and hydroxycitronellal from citronellal), and synthetic chemicals (Scheinman, 1996, De Groot *et al*, 1997).

The importance of fragrance components as allergens in specific topical pharmaceutical products has not been reported before.

The aims of this retrospective study were:

(1) To determine which and how many topical pharmaceutical products marketed in Belgium contain fragrance components.

(2) To examine the nature of the fragrance allergens in specific pharmaceutical products that have caused iatrogenic contact dermatitis.

Materials and Methods

This was a cross-sectional study for which all data were retrieved from and evaluated with in-house developed databases in our contact allergy unit (Dooms-Goossens *et al*, 1980; Goossens *et al*,

1998). The database on topical pharmaceutical products, first developed in 1978, contains information on the complete composition (including fragrance ingredients) of 3820 products marketed in Belgium (preparations by pharmacists not included) in which the nature of the fragrance allergens (as labelled) was examined. An ingredient was considered to be a 'fragrance' according to its classification as a fragrance or 'aroma' by the International Cosmetic Ingredient Dictionary and Handbook (Wenninger *et al*, 2000) (Table 1).

Table 1. Fragrance components[a] identified in 370 topical pharmaceutical products marketed in Belgium

Fragrance components	**N° of topical pharmaceutical products**
Menthol	99
Lavandula Angustifolia (lavender) Oil (Lavender oil)	68
Mentha Piperita (Peppermint) Oil (Peppermint oil)	54
"Perfume"	51
Camphor	48
Eucalyptol (Cineole/Eucalyptol)	47
Rose Flower Oil (Rose oil)	33
Citrus Medica Limonum (Lemon) Peel oil (Lemon oil)	24
Cymbopogon Nardus (Citronella) Oil (Citronella oil)	24
Pinus Sylvestris Leaf Oil (Pine needle oil)	21
Geranium Maculatum oil (Geranium oil)	20
Eucalyptus Globulus Leaf Oil (Eucalyptus oil)	17
Citrus Aurantium Bergamia (Bergamot) Fruit Oil (Bergamot Fruit oil)	17
Eugenol	15
Thymus Vulgaris (Thyme) Oil (Thyme oil)	14
Citrus Aurantium Dulcis (Orange) Flower Oil (Orange flower oil)	13
Myroxylon Pereirae (Balsam Peru) resin (Balsam peru)	13
Turpentine (Turpentine oil)	12
Rosmarinus Officinalis (Rosemary) Leaf Oil (Rosemary oil)	11
Illicium Verum (Anise) Oil (Anise oil)	11
Styrax Benzoin Gum (Benzoin)	10
Thymol	9
Colophonium	9
Benzyl alcohol	9
Anethole (Anethol)	9
Vanillin	8
Terpineol	7
Myristica Fragrans (Nutmeg) Kernel Oil (Nutmeg oil)	6
Musk Ketone	6
Linalool	6
Citrus Aurantium Amara (bitter orange) Oil (Neroli oil or Petitgrain Oil Organic)	6
Benzyl acetate	6
Melaleuca Quinquenervia oil (Niaouli oil)	5
Linalyl acetate	5
Hydroxycitronellal	5

Dihydrocoumarin	5
Cinnamyl alcohol	5
Mentha Viridis (Spearmint) Leaf Oil (Spearmint oil)	4
Melaleuca Leucadendron Cajaputi Oil (Cajeput oil)	4
Benzyl benzoate	4
Melaleuca Leucadendron Cajaputi Oil (Cajeput oil)	4
Origanum Majorana Leaf oil (Sweet marjoram oil)	3
Melissa Officinalis (Balm Mint) Leaf Oil (Balm mint oil)	3
Citrus Nobilis (Mandarin Orange) Peel oil (Mandarin oil)	3
Isoamyl Acetate (Isoamyl ethanoate)	3
Cupressus Sempervirens Oil (Cypress oil)	3
Cinnamomum Cassia Leaf (cinnamon) Oil (Cinnamon oil)	3
Cedrus Atlantica (Cedarwood) Bark Oil (Cedarwood oil)	3
Shorea Robusta Resin (Damar)	2
Cymbopongon Schoenanthus oil (Lemongrass oil)	2
Laurus Nobilis oil (Laurel oil)	2
Foeniculum vulgare (Fennel) oil (Fennel oil)	2
Coriandrum Sativum (Coriander) Fruit Oil (Coriander oil)	2
Benzyl cinammate	2
Cinnamal	1
Melaleuca Alternifolia (tea tree) Leaf Oil (Tea tree oil)	1
Jasminum Officinale (jasmine) Oil (Jasmine oil)	1
Sassafras Officinale Root Oil (Sassafras oil)	1
Santalum Album (Sandalwood) Oil (Sandalwood oil)	1
Hyssopus Officinalis Leaf oil (Oil of hyssop)	1
Dipentene (Limonene)	1
Heliotropine (Piperanol)	1
Canarium Luzonicum Gum Oil (Canarium Luzonicum- Elemi)	1
Ocimum Basilicum (Basil) oil (Basil oil)	1
Anise alcohol	1
Amyl Cinnamal	1

[a]The fragrance components are named here with the International Nomenclature of Cosmetic Ingredients (which is not the case on the labels, on which many different names for the same components could be used) as well as the technical/ trade name in bracket.

The patient database contains information (including sex, localization of the lesions, the topical pharmaceutical products used, the results of patch tests, and the sensitization sources) of 18 960 patients investigated for contact allergy between January 1978 and June 2008. They were patch tested with the European baseline patch test series (Trolab, Hermal, Reinbeck, Germany), additional series, and individual substances when indicated. In order to detect the allergenic culprits in patients suspected of iatrogenic contact dermatitis, whenever possible, the topical pharmaceutical products used along with their ingredients were tested as well. The subjects included in this study were those who presented with iatrogenic contact dermatitis from a specific pharmaceutical product and for whom a positive reaction was found to a fragrance allergen present.

The patch tests were administered with Van der Ben ^{TM} patch-test chambers (Van der Bend,

Brielle, the Netherlands), applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Fixomull® (Beiersdorf AG, Hamburg, Germany) and later on with Mefix1 (Molnlycke Health Care, Goteborg, Sweden) as adhesive tape. The patch test readings were per- formed according to the international guidelines by the International Contact Dermatitis Research Group (Wahlberg, 2001) after 2, 3 (exceptionally), and 4 days and sometimes later.

Results

A total of 18 960 patients were investigated for contact allergy, that is 12 419 (65.5%) women and 6541 (34.5%) men, for whom the MOAHLFA index (Schnuch *et al*, 1997; Uter *et al*, 2008) was: M (male), 34.5%; O (occupational dermatitis), 19.7%; A (atopic dermatitis), 22.9%; H (hand dermatitis), 40.2%; L (leg dermatitis), 5%; F (face dermatitis), 32.4%; and A (40 years and older), 43.7%. At least one of the allergens tested produced a positive reaction in 10 249 (54%) patients, that is 6103 (60%) women and 4146 (40%) men. Of 10 249, 3378 (33%) suffered from iatrogenic contact dermatitis, that is 2268 (67%) women and 1110 (33%) men. One hundred and twenty-seven patients, that is 92 (72%) women and 35 (28%) men, reacted to 48 specific topical pharmaceutical products in which fragrance components were identified as relevant allergens. Legs (n= 57), hands (n= 39), and face (n= 28) were the most commonly affected body sites in these patients, particularly in women.

We identified 66 different fragrance components (Table 1) in 370 (10% of the total) topical pharmaceutical products marketed in Belgium. Table 2 lists the nature of 48 specific products that have caused iatrogenic contact dermatitis in 127 patients, along with the nature of the 38 different fragrance components these products contain. These are products with wound healing, antiseptic or antimicrobial properties, non-steroidal anti- inflammatory drugs (NSAIDs), antihaemorrhoidal preparations, and even corticosteroid-containing medications [e.g. Mycolog® (Triadcortyl® in the UK) (Sanofi-Aventis, Diegem, Belgium), Scheriproct® and Ultralan® (both from Schering, Diegem, Belgium)]. So-called parapharmaceutical products, that is products not officially registered as pharmaceutical products, were excluded from this list. The fragrance allergens, for which the presence was all or not confirmed, but that produced positive patch test results in this population, along with the corresponding literature references for each compound, are listed in Table 3.

Table 2. The 48 fragrance-containing topical pharmaceutical products marketed in Belgium, found to be responsible for iatrogenic allergic contact dermatitis in 127 patients, along with their pharmacologic activity and the fragrance ingredients present[a]

Topical Pharmaceutical Product (n= nr. of pt. reacting)	Company	Application	Fragrance ingredients
Mycolog (cream)[b] (n= 34)	Sanofi-Aventis, Diegem	Antibiotic-Corticosteroid	"Perfume"
Fastum (gel) (n= 19)	Menarini, Zaventem	Anti-inflammatory (NSAID)	Lavender oil, neroli oil
Flexium (cream) (n= 9)	Melisana, Brussels	Anti-inflammatory (NSAID)	Benzyl alcohol, eucalyptus oil, pine needle oil
Dermophil indien (ointment) (n= 5)	Couvreur, Brussels	Wound healing	Myroxylon pereirae, rose oil
Hac (solution) (n= 5)	SSL Healthcare Belgium, Grand-Bigard	Antiseptic-disinfectant	Benzyl benzoate, terpineol
Cicatrisan (ointment) (n= 4)	Unda, Brussels	Wound healing	Myroxylon pereirae
Calendula (ointment) (n= 4)	Unda, Brussels	Wound healing	Rose oil
Homeoplasmine (ointment) (n= 3)	Unda, Brussels	Wound healing	Benzoin, benzyl alcohol
Newderm (ointment) (n= 3)	Wolfs, Sint-Niklaas	Wound healing	Geranium oil
Polyseptol (ointment) (n= 3)	Qualiphar, Bornem	Antibiotic	Bergamot fruit oil, geranium oil
Borostyrol (solution) (n= 2)	A.C.P., Brussels	Wound healing	Benzoin, bergamot fruit oil, menthol, thymol
Phenergan (cream) (n= 2)	Sanofi-Aventis, Diegem	Antihistaminic	Lavender oil
Reparil (gel) (n= 2)	Madaus, Brussels	Anti-inflammatory, Vascular disorders	Lavender oil, neroli oil
Madecassol (cream) (n= 2)	Bayer, Brussels	Wound healing	Geranium oil, lavender oil
Anusol (ointment) (n= 2)	Pfizer Consumer Health, Brussels	Anti-hemorrhoids	Myroxylon pereirae
Hibitane (cream) (n= 2)	SSL Healthcare Belgium, Grand-Bigard	Antiseptic-disinfectant	Pine needle oil
Oxyplastine (ointment) (n= 1)	Bournonville Pharma, Brussels	Wound healing	Myroxylon pereirae
Murazyme (ointment) (n= 1)	Grünenthal, Sint-Stevens-Woluwe	Wound healing	Lavender oil
Biogaze HN (bandage) (n= 1)	OJG Cons Care, Sint-Martens-Latem	Wound healing	Niaouli oil
Vitamorrhuine (ointment)* (n= 1)	PCB	Wound healing	Bergamot fruit oil, lavender oil
Vegebom (ointment) (n= 1)	Bournonville Pharma, Brussels	Wound healing	Cajeput oil, cedarwood oil, laurel oil, nutmeg oil, turpentine

			oil
Groene Duivel (plaster) (n= 1)	Colin, Blegny	Wound healing	Colophonium, turpentine
Reumatrix (plaster) (n= 1)	Beiersdorf, Anderlecht	Wound healing	Colophonium
Baume Contre Brulures (ointment)* (n= 1)	Qualiphar, Bornem	Wound healing	Myroxylon pereirae
Advantan (cream) (n= 1)	Schering, Diegem	Corticosteroid	Benzyl alcohol
Amicla (cream) (n= 1)	ERFA, Etterbeek	Corticosteroid	Benzyl alcohol
Dermaspray (solution) (n= 1)	Roche, Anderlecht	Antiseptic-disinfectant	Benzyl alcohol
Neo-Sabenyl (solution) (n= 1)	Qualiphar, Bornem	Antiseptic-disinfectant	Lavender oil
Dettol (solution) (n= 1)	Reckitt Benckiser, Brussels	Antiseptic-disinfectant	"Perfume", terpineol
Reflex (gel) (n= 1)	Boots, Louvain la Neuve	Anti-inflammatory (NSAID)	Benzyl alcohol, menthol
Nifluril (ointment) (n= 1)	Bristol-Myers Squibb Belgium, Waterloo	Anti-inflammatory (NSAID)	"Perfume"
Lotriderm (cream) (n= 1)	Schering Plough, Brussels	Anti-mycosis	Benzyl alcohol
Mycospor (cream) (n= 1)	Bayer, Brussels	Anti-mycosis	Benzyl alcohol
Pevaryl (cream) (n= 1)	Janssen-Cilag, Berchem	Anti-mycosis	Rose oil
Chloramphenicol (cream) (n= 1)	SKF-Rit, Rixensart	Antibiotic	Neroli oil
Sarnol (lotion) (n= 1)	Stiefel, Herverlee	Anti-pruritus/Analgesic	Camphor, menthol, "perfume"
Vicks Vaporub (n= 1)	Procter & Gamble, Stombeek-Bever	To inhale (to relieve symptoms of bronchitis, sinusitis, etc.)	Camphor, eucalyptus oil, menthol, nutmeg oil, thymol, turpentine
Duofilm (solution) (n= 1)	Stiefel, Heverlee	Keratolytic	Colophonium
Scheriproct (ointment) (n= 1)	Schering, Diegem	Analgetic, antinflammatory	Cypress oil
Caladryl (ointment) (n= 1)	Omega Pharma, Nazareth	Anti-pruritus	Lavender oil
Benzoeoplossing (solution)[c] (n= 9)	ABL	Degreaser	Benzoin, benzyl alcohol, rose oil
Maturosan (ointment)[c] (n= 4)	Sam	Wound healing	Benzoin, benzyl alcohol, elemi resin, turpentine, turpentine oil
Madecassol (ointment)[c] (n= 3)	Laroche Navarron, Brussels	Wound healing	Geranium oil, lavender oil
Pyal (ointment)[c](n= 3)	Sanico, Turnhout	Antibiotic	Bergamot fruit oil, lavender oil
Oxyplastine (ointment)[c] (n= 2)	Promedy	Wound healing	Geranium oil, lemongrass oil, myroxylon pereirae, origanum

			oil, thyme oil
Ultralan Vet Gras (ointment)[c](n= 1)	Schering, Diegem	Corticosteroid	Bergamot fruit oil, cinnamyl alcohol, citronella oil, piperonal, hydroxycitronellal, ionone, lavender oil, linalool, musk ketone, neroli oil
Desinfecterende en helende zalf (ointment)[c] (n= 1)	Unda, Brussels	Antiseptic-disinfectant	Myroxylon pereirae
Inhalene (solution)[c] (n= 1)	Alcon, Breendonk	Cold symptoms, laryngitis, sinusitis	Cajeput Oil, cypress oil, eucalyptol, eucalyptus oil, musk ketone, lavender oil, peppermint oil, pine needle oil

[a] During the period 1978-2008, the names of the manufacturers or the companies marketing these topical pharmaceutical products, as well as the content of the products may have changed over the years.

[b] Mycolog® cream is marketed in the U.K. as Triadcortyl®.

[c] Some of them are no longer commercially available, hence no companies in Belgium anymore.

Table 3. The fragrance allergens identified in 127 patients reacting to the 48 specific topical pharmaceutical products

Allergens	**PC**	**PNC**	**Litertature references**
Fragrance-mix I	36	51	26, 28, 42, 63, 71-75
Cinnamyl Alcohol	2	4	64, 71,73
Cinnamal	2	3	
Geraniol[a]	4	2	74-77, 80
Hydroxycitronellal[a]	3		64
Oak moss (*Evernia prunastri*)[a]		10	
Eugenol[a]	4	2	81
Iso-eugenol[a]	3	3	
Fragrance Mix II	2	1	
Citral[a]	1	1	
Citronellol[a]	2		
Myroxylon Pereirae Resin (balsam of Peru)	21	38	25-28, 42, 57
Colophonium	7	14	42
Essential Oils			11, 12
Lavender Oil	24	2	12, 48, 75, 78
Neroli Oil	14		48, 12
Eucalyptus Oil	10		12
Cananga Odorata Flower (ylang ylang) Oil		2	12
Cinnamon Oil		1	48
Jasmine Oil		1	11, 12
Geranium Oil	12		11, 12
Laurel Oil	2		12, 53-55
Niaouli Oil		2	3

Pine Needle Oil	4		32, 35
Tea Tree Oil	2		
Lemon Oil		1	
Rose Oil	5		
Other allergens			
Perfume (Mycolog®)	34		23, 24, 64, 79
Perfume (Nifuril®)	1		
Amyl Cinnamic Alcohol[a]	2	1	
Thymol	1		
Camphor	2		
Limonene	7	2	
Tree moss (*Evernia furfuracea*)		1	
Styrax Benzoin Gum (Benzoin)	19		60, 61
Benzyl Alcohol	5		49, 58, 64
Benzyl Benzoate	1		
Terpineol	5	1	34
Turpentine	2		

FM I, fragrance mix I; FM II, fragrance mix II; PC**,** presence confirmed in the causal pharmaceutical product; PNC, presence not confirmed.

[a] These allergens were not listed in Table 1 since they were tested due to their presence in FM I or in FM II, or in Mycolog® "perfume". However, all patients were not tested with all individual components.

With regard to the fragrance allergens identified among the 127 patients, fragrance mix I (FM I) [containing cinnamyl alcohol, cinnamal, eugenol, amyl cinnamal, hydroxycitronellal, geraniol, iso- eugenol, and *Evernia prunastri* (oakmoss) absolute] was the most frequent allergen found in the European baseline patch test series, that is 87 reactions (Table 3), followed by *M. pereirae* with 59 positive reactions, both reacting concomitantly in 40 cases. To a lesser extent, colophonium was found with 21 reactions, reacting concomitantly to FM I and *M. pereirae* in 11 cases and with FM I alone in 4 more cases.

Since 2005, a mixture of six additional fragrance materials has been available for introduction into the baseline series, that is fragrance mix II (FM II) (containing hydroxyisohexyl 3-cyclo-hexene carboxaldehyde, citral, citronellol, farnesol, coumarin, and hexyl cinnamal) (Frosch *et al*, 2005a; Frosch *et al*, 2005b), for which we detected three relevant positive reactions. Two patients reacted to citronellol, and the third patient reacted to farnesol, for which no relevant explanation was found, and to eucalyptus oil (cf. below) present in Flexium® gel (Melisana, Brussels, Belgium). All three also reacted to FM I, one of whom to cinnamyl alcohol present in it.

Forty-five patients presented with 82 reactions to one or more essential oils (Table 3); seven of them did not react to FM I, *M. pereirae*, or colophonium. In addition, in those subjects who were tested with some of the individual ingredients of essential oils, we found following positive reactions: three to (oxidized) limonene in patients positive to lavender oil and two to geraniol in patients reacting to geranium oil; of five patients reacting to rose oil, one had a positive reaction to benzyl alcohol, one to citronellol, one to limonene, and one to geraniol. The reactions to citronellol

(as a component of FM II, cf. above) were relevant too for contact allergy to rose and geranium oil, present in Calendula1 ointment (Unda, Brussels, Belgium) and Polyseptol® ointment (Qualiphar, Bornem, Belgium), respectively.

There were 34 patients who reacted to the perfume present in Mycolog cream. According to data obtained from the company, this perfume contains 28 ingredients, among which one (hydroxycitronellal) and three (citral, citronellol, and coumarin) are also present in FM I and FM II, respectively. Not all patients reacting to this perfume were tested with the fragrance mixes or with other fragrance ingredients, but of the six tested, one presented with a positive reaction to citral, three to amylcinnamal, two to limonene, and one to benzyl alcohol. Two subjects also reacted to lavender oil, which, besides limonene (not tested in these cases), might share also other common ingredients with lavandin oil (*Lavandula Hybrida*) present in Mycolog cream.

We found 19 patients with positive reactions to benzoin, 7 of them because of a degreasing solution, 5 to wound healing preparations, and 7 to an ointment preparation against fissures. Of 19, 9 presented simultaneous reactions to both FM I and *M. Pereirae* and another 4 to FMI, *M. pereirae*, and colophonium.

Simultaneous reactions to *M. pereirae* and propolis were found in 5 patients and to the fragrance components of FM I and *M. pereirae* (one of them also to colophonium) and Compositae mix (Trolab, Hermal) in 7 patients, of whom 6 reacted to arnica and 2 to sesquiterpe-lactone mix.

If only the baseline series had been tested, fragrance allergy would have been missed in 24 of 127 (19%) patients reacting to the 48 responsible products. They did react to perfume, terpineol, geranium oil, lavender oil, benzoin, pine needle oil, laurel oil, bitter orange oil, rose oil, laurel oil (oxidized), limonene, or thymol.

Figs 1 and 2 illustrate allergic contact dermatitis from HAC® solution (SSL Healthcare, Groot-Bijgaarden, Belgium) and the positive test to its ingredient a-terpineol. Fig. 3 shows residual permanent hyperpigmentation as a complication of allergic contact dermatitis from benzoin in a degreasing solvent applied under a cast. Besides benzoin, the patient also reacted to FM I and *M. pereirae*.

Fig. 1. Allergic contact dermatitis from terpineol in HAC® solution (SSL Healthcare, Groot-Bijgaarden, Belgium).

Fig. 2. Positive reaction to terpineol 5% petrolatum in the same patient.

Fig. 3. Residual permanent hyperpigmentation as a complication of allergic contact dermatitis from benzoin tincture in a degreasing solvent applied under a cast.

Discussion

Most patients reacting to 48 specific fragrance-containing topical pharmaceutical products reacted to FM I, followed by *M. pereirae*, and colophonium, the fragrance-allergy markers present in the European baseline patch test series. Reactions to them were often associated (Wohrl *et al*, 2001; Nardelli *et al*, 2008; Thyssen *et al*, 2008; Hjorth 1961; Larsen, 1977), which is explained by the presence of similar components. In 1977, Larsen even introduced FM I partly based on positive patch test results obtained with the fragrance components of Mycolog perfume (Larsen, 1977; Larsen, 1985) to detect fragrance allergy.

Allergic contact dermatitis from *M. pereirae* in Dermophil Indien (Couvreur, Brussels, Belgium) was described by Foussereau (Foussereau, 1975) in 1975, and recent studies (Avalos-Peralta *et al*, 2005; Machet *et al*, 2004; Green *et al*, 2007) indicate that the increased incidence of sensitization to *M. pereirae* observed is mainly because of fragrances present in topical medications for wound-healing or antimicrobial effects (Hasan *et al*, 2005; Lindberg *et al*, 2007). Most ingredients of *M. pereirae* are present in or related to other allergenic fragrance materials, for example three of them being present in FM I, that is cinnamyl alcohol, cinnamal, and eugenol. Furthermore, propolis, a resinous substance collected by bees (Martindale, 1999), to which anti-inflammatory and antimicrobial properties are attributed, also shares several minor sensitizers with M. pereirae, which explains the concomitant reactions; according to Wohrl et al. (Wohrl *et al*, 2001), it is also a helpful indicator for fragrance allergy.

Colophonium may cross-react or co-react with other fragrance allergens (Saap *et al*, 2004; Roesyanto *et al*, 1990; Malten *et al*, 1976; Iorizzo *et al*, 2000; Johansen *et al*, 2002; Lepoittevin *et al*, 2000) that also contain oxidized terpenes (Karlberg *et al* 1988; Karlberg, 1991); it is mostly found as an allergen in classical adhesive tapes (not taken into account in this study) but is also used as a vehicle component in wart medications, surgical paints (Reichert-Penetrat *et al*, 2001), and Chinese herbal medicine (Li, 1995; Chen *et al*, 2003), the latter of which two such cases were included here. Such medications generally contain more fragrance allergens and plant extracts than topical pharmaceutical products sold in Europe (Chen *et al*, 2003). Moreover, modified colophonium is also present in wound dressings.

Essential oils are mainly constituted of terpenes such as a-pinene and b-pinene, citral, geraniol, linalool, citronellal, hydroxycitronellal, and limonene but also of other organic chemical com- pounds, including aromatics, aliphatics, alicyclics and heterocyclics. It has been shown that terpenes are not allergenic themselves but that their oxidation products are the strongest allergens

formed being mainly hydroperoxides (Matura *et al*, 2003; Sköld *et al*, 2002; Sköld *et al*, 2004; Sköld *et al*, 2008). Although patch test material of the oxidized forms of linalool and limonene are not commercially available yet (Sköld *et al*, 2002; Sköld *et al*, 2004), we did test with aged materials and thus obtained positive patch test results. As shown in Table 2, essential oils are present in several pharmaceutical topical products, particularly in those that have been marketed several decades ago. Unfortunately, they are also found in more recently marketed preparations such as NSAIDs. In a recent study performed in our department (Devleeschouwer *et al*, 2008), 10 of 40 patients with positive photo-patch tests to Fastum® (Menarini, Zaventem, Belgium) presented with a contact allergy to the essential oils present in them. Moreover, 22 of 40 reacted to FM I and/or *M. pereirae* but not to the essential oils contained in these products. In agreement with the literature, fragrance allergy, and cinnamyl alcohol in particular, is indeed often related to photocontact allergy to ketoprofen, the reason for which is not clear (Matthieu *et al*, 2004; Girardin *et al*, 2006).

Because of their pharmacological properties, such as antiseptic, analgesic, or anti-inflammatory effects, essential oils and their ingredients are often used for medical instead of cosmetic reasons. Currently, these substances are frequently found in aromatherapy (Cockayne *et al*, 1997; Trattner *et al*, 2008; Keane *et al*, 2000) and are applied directly to the skin for the treatment of, for example, arthritis (Ozden *et al*, 2001; Onder *et al*, 2003), muscular pains (Adisen *et al*, 2007), etc. In dentistry, for example, eugenol, an important chemical constituent of clove oil, is widely used because of its antiseptic properties (Estlander *et al*, 2006). A Swedish study has reported that even *M. pereirae*, which also contains eugenol, is used in dental cement and indicated that the oral mucosa might also be a sensitization site (Svedman *et al*, 2007).

Benzyl alcohol (Curry *et al*, 2005; Sestini *et al*, 2004) is present in *M. pereirae* at 1–2% concentration and is an aromatic preservative agent that also has anaesthetic, antipruritic, and viscosity-decreasing properties (Curry *et al*, 2005; Sestini *et al*, 2004; Hausen *et al*, 2001). Despite its widespread use, sensitization to this allergen is considered rare but has been responsible for allergic contact dermatitis from antibacterial and antimycotic creams, topical corticosteroids, sunscreens, analgesic sprays, and sclerosing agents (Curry *et al*, 2005; Sestini *et al*, 2004).

Benzoin is a balsamic resin obtained from *Styrax* benzoin Dryander and other species of *Styrax* (*Faro*. Styraceae). The tincture is used (Lakshmi *et al*, 2006; Scardamaglia *et al*, 2003) to enhance the adhesive properties of tape and bandages, as an antiseptic, and also as a solvent for drugs, for example in podophyllin tincture, used in the treatment of venereal warts (Lakshmi *et al*, 2006). The main constituents of benzoin are resin, benzoic acid, and cinnamic acids and their esters. In addition, traces of vanillin, benzaldehyde, styrol, and styracin are also present (Lakshmi *et al*, 2006; Scardamaglia *et al*, 2003). Cross-reaction between benzoin and similar allergens (FM I, *M.*

pereirae, colophonium, and tea tree oil) have been reported (Lakshmi et al, 2006; Scardamaglia et al, 2003).

The observation of simultaneous reactions to fragrances and Compositae plant extracts (sesquiterpene lactones) can be explained by the common presence of terpenes (Paulsen *et al*, 2005; Paulsen *et al*, 2002).

In agreement with the literature, multiple sensitization to several fragrances was also observed in this study population, and the risk becoming higher because of the frequent application of topical pharmaceutical products also in chronic dermatological conditions in which the skin barrier is damaged: leg ulcer patients, patients with dermatitis on the hands (also direct contact when applying the products on the skin), patients with chronic facial dermatitis such as seborrhoeic or atopic dermatitis (Avalos-Peralta *et al*, 2005; Machet *et al*, 2004; Uter *et al*, 2002; Zmudzinska *et al*, 2006; Lim *et al*, 2007).

Since 1997, all cosmetic ingredients have been labelled in a uniform manner with the International Nomenclature of Cosmetic Ingredients name, except for fragrance. Since 2005, the EU has also required that 26 important fragrance allergens be labelled specifically (EU Directive, 2003). Moreover, restrictions have been made on the use concentrations of certain allergens, for example isoeugenol (Rastogi *et al*, 2008). Besides EU regulations, the International Fragrance Research Association gives advice as to the use of certain fragrance allergens by the perfume industry; for example, the use of crude *M. pereirae* in cosmetics has been banned since 1982 (Api *et al*, 2006) (albeit that the extracts or distillates are not necessarily less allergenic). In contrast, in topical pharmaceutical products in Europe, there is no legislation regarding such labelling: the nomenclature of the ingredients has not been standardized (Degreef *et al*, 1978), and several synonyms may appear on the label or on the notice. As to the presence of fragrance allergens, no restrictions have been made either.

Conclusions

The fragrance-allergy markers in the baseline series did pick up most of the patients sensitized to fragrance allergens present in the 48 specific topical pharmaceutical products to which they reacted. However, extended patch testing with the products used by the patient, along with the ingredients, is also needed because one fifth of them only reacted positively to an essential oil or to another fragrance allergen present. The possibility of testing all the components of a commercial product depends, of course, on the manufacturer's goodwill to provide all ingredients.

Because topical pharmaceutical products are applied on more vulnerable, and often diseased skin (wounds, leg ulcers, eczema, etc.), they thus constitute another important source of fragrance

sensitization, which predisposes the patients to develop multiple sensitivities and to react also to fragrance-containing cosmetics (and other fragranced materials). Besides their use as a pharmacologically active ingredient in some cases, the use of fragrances in topical pharmaceutical products is unnecessary. In agreement with other authors (Schliemann *et al*, 2006; Garioch *et al*, 1989), we also urge for better legislative measures and a standardized labelling of all ingredients of such products.

Chapter 8

Fragrance allergens in ‘specific’ cosmetic products

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Contact Dermatitis 2011, 64, 212–219

Summary

Background. Together with preservative agents, fragrance components are the most important sensitizing culprits in cosmetic products.

Objectives. To identify the nature of the fragrance ingredients responsible for allergic contact dermatitis (ACD) from specific cosmetic products.

Methods. Between 2000 and 2009, positive patch test reactions or positive usage tests with the patients own cosmetic products, were recorded using a standardised form. *Results*. Of the 806 cosmetic records, corresponding to 485 patient files, 344 concerned reactions to fragrance ingredients that according to the label were present ('Presence Confirmed' [PC n = 301]) or suspected to be present ('Presence Not Confirmed' [PNC n = 376]) in the causal cosmetic products used, which belonged to 15 different categories, toilet waters/fine perfumes being the most frequent. Geraniol in fragrance mix I (FM I) and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) in FM II were the most frequent PC, and together with hydroxycitronellal and *Evernia prunastri* (oak moss) the most frequent PNC ingredients in the causal cosmetic products. Limonene was the most frequent PC confirmed fragrance allergen.

Conclusions. This study not only underlines the usefulness of fragrance-ingredient labelling in order to identify the causal allergen(s) present in specific cosmetic products, but may also provide information on trends in the actual use of sensitizing fragrance ingredients in them.

Introduction

Together with preservatives, fragrance chemicals are the most important sensitizers in cosmetic products (Wetter *et al*, 2010). Notwithstanding the many publications on this subject, the specific fragrance allergens contained in the different types of cosmetic products causing allergic contact dermatitis (ACD) have been infrequently identified (Goossens, 2006).

In the United States, cosmetic ingredients have been listed on the packaging of cosmetic products for over 20 years, but in Europe this has been done only since 1997, with fragrance components requiring to be labelled as 'perfume' or 'aroma' (EU Directive, 2003) (the word 'fragrance' has not been mentioned in the legislation, although is frequently used on the packaging of cosmetic products). A later amendment to the European Cosmetics Directive in March 2005 stated that manufacturers had to individually label 26 recognized fragrance sensitizers (EU Directive, 2003), using the International Nomenclature Cosmetic Ingredient (INCI) name, which is based on the US Cosmetic, Toiletry, and Fragrance Association nomenclature (Wenninger *et al*, 2000). Labelling is required if such a component is present at ≥10 ppm in leave-on cosmetic products or ≥100 ppm in rinse-off cosmetic products.

The present study sought to identify the nature of fragrance allergens responsible for ACD caused by specific cosmetic products. It does not, of course, reflect the most frequent fragrance allergens encountered in the total population tested.

Materials and Methods

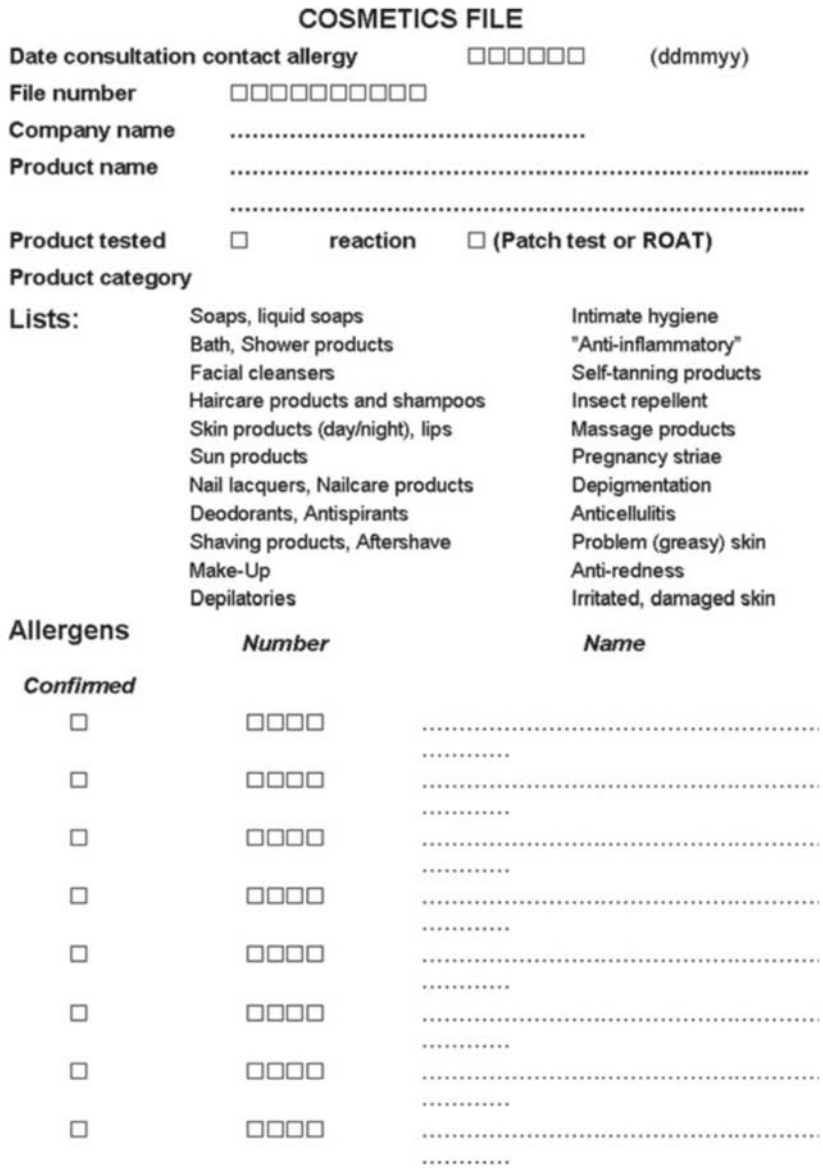
This was a cross-sectional study, for which all data were retrieved from, and evaluated with, databases developed in-house in our department (Goossens *et al*, 1998).

Between January 2000 and September 2009, positive patch test reactions or positive usage test results with the patients' own cosmetic products, as well as allergic
contact reactions to specific cosmetic ingredients, were recorded with the use of a standardized form (Fig. 1). When, according to the label, the 'specific' allergens to which the patients were sensitized were identified in the causal cosmetic product used, the allergens were indicated with 'presence confirmed' (PC), and for 'specific' allergens that were only suspected to be present, the indication 'presence not confirmed' (PNC) was used. In the latter case, the patients had not kept the packaging with the label, and, in the case of fragrances, were using products for which only the term 'fragrance' or 'perfume' had been mentioned (before 2005). A reaction to a specific fragrance allergen shown to be present in the causal product did not imply, of course, that only this allergen was held to be responsible for the dermatitis in cases where the patient reacted to another ingredient as well.

All subjects had been tested with the European baseline series containing fragrance mix I (FM

I), Myroxylon pereirae, colophonium, and, from 2005 onwards, fragrance mix II (FM II) (Bruze *et al*, 2008). The patients reacting to FM I and FM II were, in most cases, tested with the individual ingredients, and some of them were also tested with other fragrance components.

All patch test results had been obtained with Van der Bend® patch test chambers (Van der Bend, Brielle, The Netherlands) applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Mefix® (Mölnlycke Healt Care, Göteborg, Sweden) as adhesive tape. The patch test readings were performed according to the international guidelines of the International Contact Dermatitis Research Group (Wahlberg, 2001) after 2 days, 3 days (exceptionally), and 4 days, and sometimes also later. Sometimes, repeated open application tests or usage tests had been performed if a cosmetic product that remained negative on patch testing was suspected to be responsible for the dermatitis that the patient had presented with.



COSMETICS FILE

Date consultation contact allergy ☐☐☐☐☐☐ (ddmmyy)

File number ☐☐☐☐☐☐☐☐☐☐

Company name .....

Product name .....

.....

Product tested ☐ reaction ☐ (Patch test or ROAT)

Product category

Lists:

Soaps, liquid soaps	Intimate hygiene
Bath, Shower products	"Anti-inflammatory"
Facial cleansers	Self-tanning products
Haircare products and shampoos	Insect repellent
Skin products (day/night), lips	Massage products
Sun products	Pregnancy striae
Nail lacquers, Nailcare products	Depigmentation
Deodorants, Antispirants	Anticellulitis
Shaving products, Aftershave	Problem (greasy) skin
Make-Up	Anti-redness
Depilatories	Irritated, damaged skin

Allergens	*Number*	*Name*
Confirmed		
☐	☐☐☐	.....
☐	☐☐☐	.....
☐	☐☐☐	.....
☐	☐☐☐	.....
☐	☐☐☐	.....
☐	☐☐☐	.....
☐	☐☐☐	.....
☐	☐☐☐	.....

Fig. 1. Standardized form used to collect information on specific causal cosmetic products.

Results

Of the 806 records, corresponding to 485 patient files (some reacted to several cosmetic products), 344 concerned reactions to FM I and FM II, and/or to 28 fragrance ingredients, which, according to the label, were present in the causal cosmetic products used (PC, n= 301), or concerned an ingredient suspected to be present (PNC, n=376). Several fragrance allergens often accounted for reactions to one particular cosmetic product. A slight majority of the fragrance allergens that were only suspected to be present were found in the years 2000–2005, as the 26 individual ingredients were not required to be labelled then, that is, 219 (58%) of 376 PNC ingredients. However, many patients had still continued to use 'older' products afterwards (especially in the case of fine fragrances) or had not always kept the packaging.

Table1 gives the total number of PC and PNC ingredients of the fragrance allergens, as well as their presence in the different types of cosmetic product found to be responsible for ACD in the patients investigated.

Table 1. Number of 'presence confirmed' (PC) and 'presence not confirmed' (PNC) fragrance allergens in 'specific' causal cosmetic products to which the patients studied reacted positively

Allergen	Eau de toilette (n = 103)	Bath and shower (n = 23)	Toilet soap (n = 10)	Hair care (n = 30)	Skin care (n = 88)	Sun products (n = 14)	Toothpaste (n = 2)	Face cleansers (n = 5)	Deodorants (n = 46)	Shaving products (n = 16)	Depilating products (n = 1)	Makeup (n = 1)	Intimate hygiene (n = 2)	Massage products (n = 2)	Self-tanning products (n = 1)	PC total	PNC total
FM I*	8 (55)	2 (3)	(1)	3 (2)	4 (29)	1 (5)	—	(1)	1 (13)	3 (7)	—	—	1	(1)	—	23	117
FM II*	11 (10)	3 (2)	2 (1)	11	10 (11)	1 (2)	—	2	3 (2)	—	—	(1)	1	—	—	44	29
Cinnamyl alcohol	2 (2)	1	—	(1)	—	—	—	—	(2)	—	—	—	—	(1)	—	3	6
Cinnamal	—	—	—	(1)	—	—	—	—	(2)	—	—	—	1	(1)	—	1	4
Hydroxycitronellal	4 (17)	—	—	—	3 (6)	(3)	(1)	—	3 (6)	(1)	—	—	—	—	—	10	33
Amyl cinnamal	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—
Geraniol	5 (9)	2 (1)	1	2	7 (4)	1	—	—	(2)	—	—	—	—	—	—	18	16
Eugenol	(2)	—	—	—	(1)	1 (1)	1 (1)	—	3 (3)	(1)	—	—	—	(1)	—	5	10
Isoeugenol	2 (6)	—	—	—	(3)	—	(1)	—	(1)	(2)	—	—	—	—	—	2	13
Evernia prunastri	1 (24)	—	—	—	(3)	(1)	—	—	(5)	1 (2)	—	—	—	—	—	2	35
HICC	12 (36)	5 (1)	1 (2)	8	11 (10)	(1)	—	3	7 (15)	(7)	—	—	—	—	1	48	72
Citral	2 (3)	—	—	—	2	—	—	—	1	—	—	—	—	—	—	5	3
Farnesol	1 (4)	—	—	1	1 (1)	(1)	—	—	4 (1)	—	—	—	—	—	—	7	7
Citronellol	1 (5)	3	(1)	3	3 (1)	—	—	—	—	—	—	—	—	—	—	10	7
α-Hexyl cinnamal	1 (2)	2	—	3	—	—	—	—	1	—	—	—	1	—	—	8	2
Coumarin	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	2	—
Limonene	7 (5)	9	3	11	13 (1)	1	3	1	4 (1)	1	1	(1)	1	—	—	55	8
Linalool	—	—	—	3	4	—	—	—	3	—	—	—	—	—	—	10	—
Butylphenyl methylpropional	2 (1)	1	—	2	(2)	1	—	—	2	—	—	—	1	—	—	9	3
α-Isomethylionone	(1)	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	1
Menthol	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—
Perfume	3	3	—	2	7	1	—	1	1	3	—	—	—	—	—	21	—
Eucalyptus oil	—	1	—	—	1 (4)	—	—	—	—	—	—	—	—	—	—	2	4
Lavender oil	(2)	2	—	—	1	—	—	—	1	—	—	—	—	1	—	5	2
Neroli oil	—	—	—	—	2 (2)	—	—	—	—	—	—	—	—	—	—	2	2
Niaouli oil	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—
Orange peel oil	(2)	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	2
Peppermint oil	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—
Rose oil	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	2	—
Tea tree oil	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—
Total number	62 (186)	34 (7)	7 (11)	50 (4)	77 (72)	7 (15)	4 (1)	7 (1)	36 (53)	6 (20)	1	1 (2)	7	1 (4)	1	301	376

FM I, fragrance mix I; FM II, fragrance mix II; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.
The total numbers of PC and PNC (in parentheses) fragrance allergens found are shown, as well as their presence in the different types of cosmetic products found to be responsible for allergic contact dermatitis in the patients investigated.
*When the individual ingredients reacted positively, the results obtained with FM I and FM II were not always indicated in the files; furthermore, the ingredients of the mixes were not tested in all subjects; moreover, if a patient reacted to a specific fragrance allergen, this does not imply that it was the only allergen responsible for the allergic reaction observed to the specific cosmetic product.

With regard to the fragrance mixes in the European baseline patch test series, in the files FM I and FM II have been recorded 23 and 44 (PC) times, respectively, that is, when one of the ingredients of these mixes was indeed found to be present in the causal product. Their ingredients were suspected to be present 117 and 29 (PNC) times, respectively. However, the results obtained with FM I and FM II had not always been indicated in the files, even when an individual ingredient reacted positively; moreover, the ingredients of the mixes were not tested in all subjects.

The specific fragrance allergens correlated with 15 categories of cosmetic that produced a positive patch test reaction or usage test result, with a predominance of certain allergens in certain product categories (Table 1). Eaux de toilette/fine perfumes were the most frequent, with 62 PC and 186 PNC fragrance allergens. They were followed by: skin care products, with 77 PC and 72 PNC allergens; deodorants, with 36 PC and 53 PNC allergens; hair care products, with 50 PC and four PNC allergens; bath and shower products, with 34 PC and seven PNC allergens; shaving products, with six PC and 20 PNC allergens; sun products, with seven PC and 15 PNC allergens; and toilet soaps, with seven PC and 11 PNC allergens. Less common products were: facial cleansers, with seven PC allergens and one PNC allergen; intimate hygiene products, with seven PC allergens; toothpaste, with four PC allergens and one PNC allergen; massage products, with one PC allergen and four PNC allergens; makeup, with one PC allergen and two PNC allergens; and depilating products and self-tanning products, each with one PC allergen (Table 1).

Geraniol in FM I and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) in FM II, as well as limonene, in particular, were the most frequent ingredients for which presence could be confirmed in the causal specific cosmetic products, and, together with hydroxycitronellal and *Evernia prunastri* in FM I, were the most frequent fragrance allergens also suspected to be present.

The results for the number of cosmetic products per year and the percentages of the most common fragrance allergens are shown in Fig. 2. In addition, Fig. 3 shows the trends in frequency over the years (2000–2009) of the most common fragrance allergens, expressed as percentages.

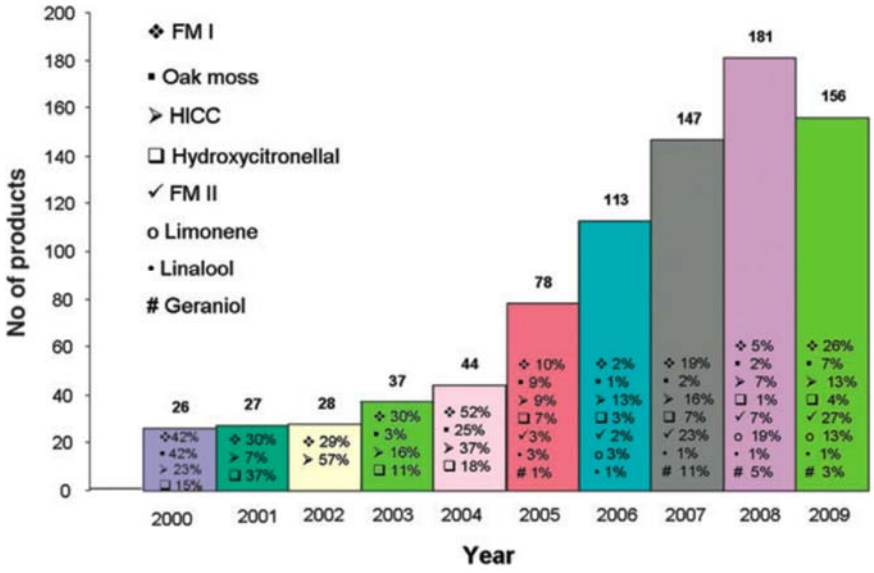


Fig. 2. Number of causal cosmetic products collected per year, along with the percentages of the most common fragrance allergens. FM I, fragrance mix I; FM II, fragrance mix II; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; Oak moss, *Evernia prunastri*.

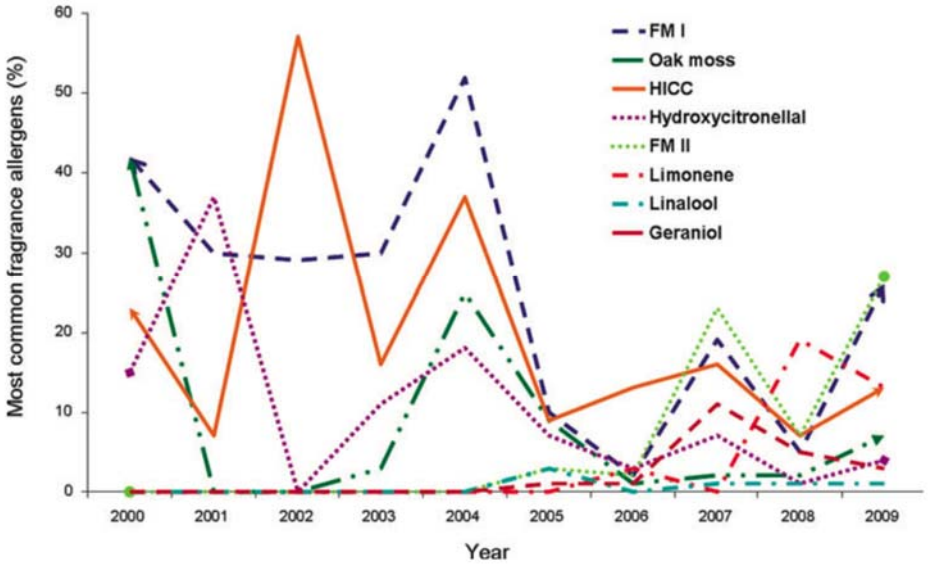


Fig. 3. Trends in frequency (%) over the years of the most common fragrance allergens included in the study. FM I, fragrance mix I; FM II, fragrance mix II; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; Oak moss, *Evernia prunastri*.

Discussion

FM I, *Myroxylon pereirae*, colophonium (although to a minor extent) and the more recently

introduced FM II (Bruze *et al*, 2008) are the diagnostic markers for perfume allergy in the baseline patch test series (Uter *et al*, 2005; Marks *et al*, 1998; Nardelli *et al*, 2008; Thyssen *et al*, 2008). Cude *M.pereirae*, as such, has not been used in perfumery since 1982, when the International Fragrance Association banned its use in fragrances (IFRA, 2008), but there is still use of the extract or distillate of *M. pereirae* (Api, 2006). *M. pereirae* and colophonium do not need to be labelled; hence, reactions to them cannot be specifically correlated with specific cosmetic products. Concerning the trends in the fragrance sensitivity rate (Figs. 2 and 3), a fluctuating trend, either increasing or decreasing, was observed for the most common fragrance allergens. However, since 2005, an increasing trend was observed for FM II, whereas in the case of FM I, a decreasing trend was observed. This fact can be explained by the change in the composition of the fine perfumes (Rastogi *et al*, 2003). This underlines the usefulness of FM II in diagnosing fragrance allergy (Frosch *et al*, 2005a; Heisterberg *et al*, 2010;Uter *et al*, 2010; Krautheim *et al*, 2010). In addition, the value of testing other allergens, such as FM II and limonene, is demonstrated: 40% of the reactions in 2009 were attributable to these.

In the current study, the fragrance allergens identified most often correlated with eau de toilette/fine perfumes, which contain higher concentrations of fragrance chemicals, as well as with skin care products (moisturizers) and deodorants. Indeed, leave-on products are more likely to cause allergic contact dermatitis than rinse-off products, such as those used for cleansing.

Among the most frequent individual allergens, we found geraniol, which has been rarely reported as a fragrance allergen, and particularly HICC, with the highest frequency from 2002 to 2004; this, as described previously, is often responsible for axillary dermatitis, because of its use in deodorants (Nardelli *et al*, 2008; Rastogi *et al*, 1998).

Limonene was also a frequent allergen identified in several cosmetic products involved, also showing an increasing trend. According to the literature (Matura *et al*, 2003), limonene and linalool, the latter having caused only a few reactions in our study, are among the most commonly found fragrance ingredients in consumer products, not only in cosmetic but also in domestic and occupational products, in particular (Matura *et al*, 2003). For example, it was shown that 97% of the deodorants analysed contained linalool, whereas it was present in 61% of the so-called household products (soaps, surface cleaners, fabric conditioners, and laundry detergents for hand washing and dish washing) investigated; 78% of the latter contained limonene (Rastogi *et al*, 2001). Furthermore, linalool was found to be present in 91% of analysed cosmetic products on the Dutch market (de Groot *et al*, 1994). Both terpenes are also common constituents of several essential oils (including rose, lavender and geranium oils) and are not allergenic by themselves, but because of their oxidation products, mainly hydroperoxides (Sköld *et al*, 2002). Although patch test material of the oxidized forms of linalool and limonene is not yet commercially available, we did test with aged

materials, and thus obtained positive patch test results. As shown in Table 1, some essential oils were responsible for reactions to several specific cosmetic products, lavender oil being the most frequent.

Restrictions on the use in cosmetic products of the specific fragrance allergens found

Restrictions on the use concentrations of certain allergens, such as isoeugenol, HICC, and *E. prunastri*, have been applied over the years. Besides EU regulations, the International Fragrance Research Association (IFRA, which provides recommendations to the perfume industry regarding the use of certain fragrance allergens, and which has guidelines on all of the 26 fragrance materials that need to be labelled), suggested in their guidelines that until May 1998, isoeugenol, for example, could safely be used at a level of 0.2% in consumer products (White *et al*, 1999), to be reduced in 1998 to 0.02% (IFRA, 2008). IFRA has since further restricted its use, and has revised the guideline on this material numerous times, most recently in 2008, when the use of isoeugenol was limited to 0.01% in category 1 (lip products) and category 2 (deodorants) (IFRA, 2008).

Chemical analysis of common cosmetic products, such as best-selling women's perfumes, natural ingredient- based cosmetics, lower-priced cosmetic products, ordinary leave-on cosmetic products, and children's cosmetics and cosmetic toys, has demonstrated the presence of isoeugenol in up to 70% of the investigated products, and at concentrations in the range 0.001 – 0.17%. Bruze et al. recommended that the concentration of isoeugenol should be lower than 0.0063% in deodorants, although studies to determine the most appropriate concentration remain to be performed (Bruze *et al*, 2005).

In the present study, isoeugenol was not a common allergen found in the causal cosmetic products, possibly as a result of such restrictions; however, isoeugenol may be substituted by isoeugenol esters, which are not labelled but end up in the skin as isoeugenol (Tanaka *et al*, 2004), for which new regulations would be appropriate.

HICC has been used in consumer products for many years, without limitations. In 2003, the EU scientific advisory committee recommended that its concentration be limited to 0.02% (200 ppm) in cosmetic products (EU Commission, 2003). However, a more recent study showed that it was found in most perfumes and at maximum concentrations exceeding the recommendation for safe usage as issued by the advisory committee, by a factor of 10 (Rastogi *et al*, 2007). A recent IFRA standard (in August 2009) recommended that the HICC concentration should be decreased to 0.02% in categories 1 (lip products and toys), 2 (deodorants and antiperspirants), and 7 (intimate and baby wipe products), and to 0.2% in products belonging to other cosmetic categories (IFRA, 2009). According to our results, HICC is still a common allergen in several cosmetic categories.

With regard to *E. prunastri*, in 2004 the EU Scientific Committee on Consumer Products

(SCCP) had also recommended that atranol and chloroatranol, the main allergens in oak moss absolute, because of their strong sensitizing potential, could not be present in any cosmetic product, and considered untreated oak moss to be unsafe for the consumer (SCCP, 2004). The most recent revision by the IFRA was issued in 2008, limiting the use of *E. prunastri* to 0.02% for category 1 and 0.03% for category 2, with atranol and chloroatranol below 100 ppm each (IFRA, 2008). In 2008, on the basis of new experimental sensitization data on chemically treated and untreated oak moss absolute samples, the SCCP was of the opinion that treatment of oak moss absolute at a laboratory scale was able to reduce the levels of atranol and chloroatranol to less than 2 ppm each (SCCP, 2008). In our study, the presence of *E. prunastri* was confirmed in only two causal cosmetic products, although it was suspected to be present in 35 products (30 of them before 2005), eaux de toilette (n = 24 PNC) being the most frequent cosmetic category. Here again, the low number of reactions observed after 2005 probably reflects its restriction.

Advice to fragrance-allergic patients

Of the 30 fragrance allergens found in our study, 18 have been required to be labelled since March 2005. However, managing patients with confirmed fragrance allergy often still proves difficult. Advising patients to use products without their specific fragrance allergen or to use those labelled as 'fragrance-free' will not necessary lead to avoidance of all contact with fragrance materials, owing to the possibility of cross-reactions to components that are not labelled or to discrepancies in the accepted definition of the term. Fragrance is defined as any substance, natural or synthetic, used solely to impart an odour to a cosmetic product. However, if a fragrance material has more than one function (e.g. benzyl alcohol as a preservative or methyl benzoate as an emollient), it could legally still be included in a fragrance-free product (Scheinman, 1999). There are also masking fragrances that may also (although rarely) produce contact allergy. Moreover, consumers may be unaware that flower (plant) extracts and flavourings are, in fact, fragrance materials, their INCI names being in Latin on the cosmetic labels (Nardelli *et al*, 2009c).

Limitations of the study

The main limitation of our study is the collection of the data: the results obtained with FM I and FM II had not always been indicated in the files, even when an individual ingredient reacted positively; moreover, the ingredients were not tested in all subjects reacting to the mixes. Other limitations of the study are that it is retrospective and that the numbers are much smaller for 2000–2004. During the first period, many cases had indeed not been carefully documented, and there is also an apparent rise in cosmetic allergy cases in our unit.

Conclusions

Labelling of the 26 individual fragrance ingredients, which has only been required since March 2005, has proven to be very useful in order to identify specific allergens in contact dermatitis patients suffering as a result of the use of fragrance-containing cosmetic products, to guide fragrance-allergic individuals who still wish to use fragrance-containing products in finding 'safer' alternatives – although this is not always easy and they are not always effective – and, as in this study, to determine the relevance of the fragrance allergens found in relation to the different categories of the causal cosmetic products. Studies of this type might also provide insights into current exposure to fragrances in cosmetic products and reflect the results of restrictions on specific ingredients. They could therefore also constitute a valuable basis for conducting further safety assessments.

Chapter 9

Results of patch testing with fragrance mix 1 and 2 and their ingredients, and *Myroxylon pereirae* and colophonium over a 21-year period

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SUMMARY

Background: The frequency of fragrance contact allergy has shown a fluctuating trend over the years.

Objectives: To describe the frequency of positive reactions to the baseline screening agents and fragrance mix (FM) 1 and 2 components, to determine trends of the latter over the years, and evaluate simultaneous reactions.

Patients and methods: This was a cross-sectional study on patch-test results of 13 332 patients from January 1990 to December 2011.

Results: Of the total population, 9.6% reacted positively to FM 1, and 6% of 3416 tested to FM 2 reacted positively. Of those tested with both, 30,4% of 349 FM 1-positive patients reacted to FM 2, and 51,7% of 205 FM 2-positives patients reacted to FM 1. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) and FM 2 were tested simultaneously in 3401 patients: 6 reacted to HICC alone. Nine hundred and forty patients were tested with FM 1 ingredients and 205 with FM 2 ingredients; *Evernia prunastri* was the most frequent FM 1 allergen, and HICC was the most frequent FM 2 allergen. Simultaneous reactions were frequently observed.

Conclusions: Fragrance-allergic subjects often show multiple positive reactions, some of which are highly significantly associated. Recently, there has been a decreasing trend in positivity for both *Evernia prunastri* and HICC, whereas a slight increase for cinnamyl alcohol has been observed.

Sensitivity to fragrance ingredients is recognized as a common and clinically important problem in Europe. It has been shown that 7-11.5 % of the individuals with eczematous skin conditions (Heisterberg *et al*, 2010-Thyssen *et al*, 2009), and at least 1-3 % of the general population, are allergic (delayed hypersensitivity) to baseline indicators of fragrance allergy (Uter *et al*, 2010). This makes contact sensitization to fragrances among the most common causes of allergic contact dermatitis, next to nickel and preservative agents. Although progress has been made in improving the safety standards of fragrances, the figures on adverse effects reported by dermatologists worldwide suggest that they are far from sufficient.

The currently used fragrance mix (FM) 1 (containing *Evernia prunastri*, isoeugenol, cinnamal, cinnamyl alcohol, hydroxycitronellal, eugenol, geraniol and α-amyl cinnamal) in the European baseline patch test series fails to detect a substantial number of clinically relevant fragrance allergies. It has been estimated that at least 15% of relevant cases of perfume allergy are not recognized by routine patch testing with FM 1 (Frosch *et al*, 2005a). However, Larsen et al. have reported that as much as 33% of fragrance sensitivity may be missed if it is used as the only test substance (Larsen *et al*, 1998), a percentage that might be even higher, as it depends on other fragrance components (and products) tested. For this reason, since 2005, a mixture of six additional fragrance materials has been commercialized for introduction into the baseline series (officially introduced in 2008) (Bruze *et al*, 2008), known as FM 2 [tested 14% in petrolatum, i.e. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), citral, farnesol, coumarin, citronellol, and α-hexylcinnamal]. These are all fragrance components with known sensitisation properties and widely used in cosmetics, household and industrial products, but also in topical pharmaceutical products (Nardelli *et al*, 2009b).

The pattern of fragrance contact allergy has changed over time because of industry developments, changing fashion trends, and regulatory interventions (Thyssen et al, 2008). The frequency of FM 1 contact allergy has shown a fluctuating trend over the years, either increasing or decreasing (Nardelli et al, 2008). In Denmark, an increase in frequency of FM 1 was observed from 1985-1986 to 1997-1998 in both men and women, followed by a decrease in 2006 (Thyssen et al, 2009). In a recent European multicentre study, FM 1 presented a wide frequency range depending on the countries studied; this ranged from 3.7% to 10.4%. The prevalence of sensitization to FM 1 was lowest in the southern countries (Italy and Spain) and Lithuania, and highest in western and central Europe (Uter et al, 2012a).

This study describes the frequency of positive reactions to FM 1 and 2, and to the individual components along with trends in their frequency over the years in our department. Furthermore, this study was also set up to evaluate associations between positively reacting FM I ingredients and *Myroxylon pereirae* (MP) and colophonium.

MATERIALS AND METHODS

Study population

This was a cross-sectional study for which all data were retrieved from, and evaluated with in-house developed databases in our Contact-allergy unit (Goossens et al, 1998). The patient database contains patients' information and results of patch tests of 13.332 patients investigated for contact allergy between January 1990 and December 2011.

Patch testing

All subjects have been tested with the European baseline series (Trolab, Hermal, Reinbeck, Germany) containing fragrance mix 1 (FM 1), balsam of Peru (*Myroxylon Pereirae)*, colophonium. Since 2002, 3927 were tested to HICC 5% pet. and from 2005 on 3416 to FM 2. The patients reacting to FM 1 and FM 2 were, in most cases, tested with the individual ingredients and some of the subjects were occasionally also tested with other fragrance components.

The patch tests were administered with Van der Bend patch-test chambers (Van der Bend, Brielle, the Netherlands) applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Fixomull® (Beiersdorf, Germany), and later on with Mefix® (Mölnlycke Health Care, Göteborg, Sweden). The patch-test readings were performed according to the international guidelines by the International Contact Dermatitis Research Group (ICDRG) (Wenninger et al, 2000) after 2 days, 3 days (exceptionally), and 4 days, and sometimes later.

Statistical analysis

The fragrance ingredients present in FM 1 and 2 were studied in an explorative way. The study of simultaneous positive reactions to FM 1 ingredients, as well as to MP and colophonium are presented in a 2x2 contingency table. As an appropriate statistical measure to compute the strength and the direction of the association, we used the odds ratio (OR), expressing the occurrence of positive reaction in one group as compared with another group, and their corresponding 95% confidence interval (CI). If the CI is different from 1, there is a significant association between the row and the column variable. This is an explorative study, therefore we are not correcting for multiple tests. The statistical analysis in the patch-tested patients was performed by using the SAS SOFTWARE SYSTEM version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the 13.332 patients who underwent patch testing between January 1990 and December 2011, 7486 or 56% patients presented with at least one positive patch test reaction. 1259 (9.6% out of 13114 patients tested) of them reacted positively to FM 1 and 205 (6% out of 3416 patients tested) to FM 2. 3380 patients were tested to both FM1 and 2: 106 (30,4%) out of the 349 positive patients to FM 1 also reacted to FM 2, while 106 (51,7%) out of 205 FM 2 positives also to FM1. 82 patients (2.09% out of 3927 tested) presented with a positive reaction to HICC (Table 1). 18 HICC-allergic patients did not react to FM 1. During the period 2005-2011, HICC and FM2 were tested simultaneously in 3401 patients: only 6 were positive to HICC alone.

Table 1. Positive reactions observed to fragrance mix (FM) 1, hydroxyiso-hexyl 3-cyclohexene carboxaldehyde (HICC) and FM 2

	Patients tested with FM 1 (*n*=13114)*	Patients tested with HICC (*n*=3927)+	Patients tested with FM 2 (*n*=3416)#
Positive reactions	1259	82	205
Percentage (%)	9.6	2.09	6

*****Between 1990-2011.
+ Between 2002-2011.
# Between 2005-2011.

Most patients underwent a full breakdown test when a positive reaction to FM1 or either FM 2 occurred. 940 patients were tested with the FM 1 ingredients: the frequency of positive reactions in descending order was: 24.6% (*n*= 230) to *Evernia prunastri*, 17% (n=160) to isoeugenol, 13.7% (n=129) to cinnamic alcohol, 12.5% (n=118) to eugenol, 7% (n=66) cinnamal, 5.5% (n=52) geraniol, 3.2% (n=30) α-amyl cinnamal and 2.6% (n=24) to hydroxycitronellal (Table 2). With the breakdown constituents of FM 2 tested in 205 subjects, HICC was by far the most common sensitizer (n=58), followed by farnesol (n=27), citral (n=23), hexylcinnamal (n=20), citronellal (n=11), and coumarin (n=9) (Table 3).

Table 2. Positive reactions to the constituents of fragrance mix (FM) 1 between 1990-2011

Constituents	Total (n=940)*	Percentage
Evernia prunastri	230	24.6
Isoeugenol	160	17
Cinnamic alcohol	129	13.73
Eugenol	118	12.6
Cinnamal	66	7
Geraniol	52	5.5
α amylcinnamic aldehyde	30	3.2
Hydroxycitronellal	24	2.6

*Nine hundred and forty patients were tested with the FM 1 ingredients.

Table 3. Positive reactions to the constituents of FM 2 between 2005-2011

Constituents	Total (n=205)*	Percentage
HICC	58	28.3
Farnesol	27	13.2
Citral	23	11.2
Hexyl cinnamal	20	9.7
Citronellol	11	5.4
Coumarin	9	4.4

HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde.
*Two hundred and five patients were tested with the FM 2 ingredients.

The results of absolute numbers in frequency of the different ingredients of FM 1 (1990-2011) and FM 2 (2005-2011) obtained over the years are given in Figures 1 and 2, respectively.

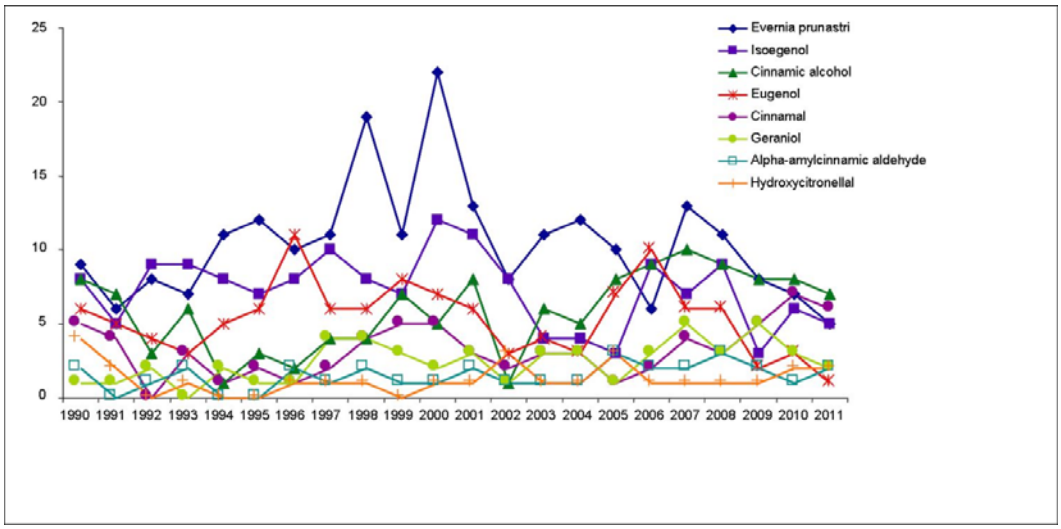

Figure 1. Evolution of the absolute numbers in frequency over the years of positive reactions to the different ingredients of fragrance mix 1 (1990-2011).

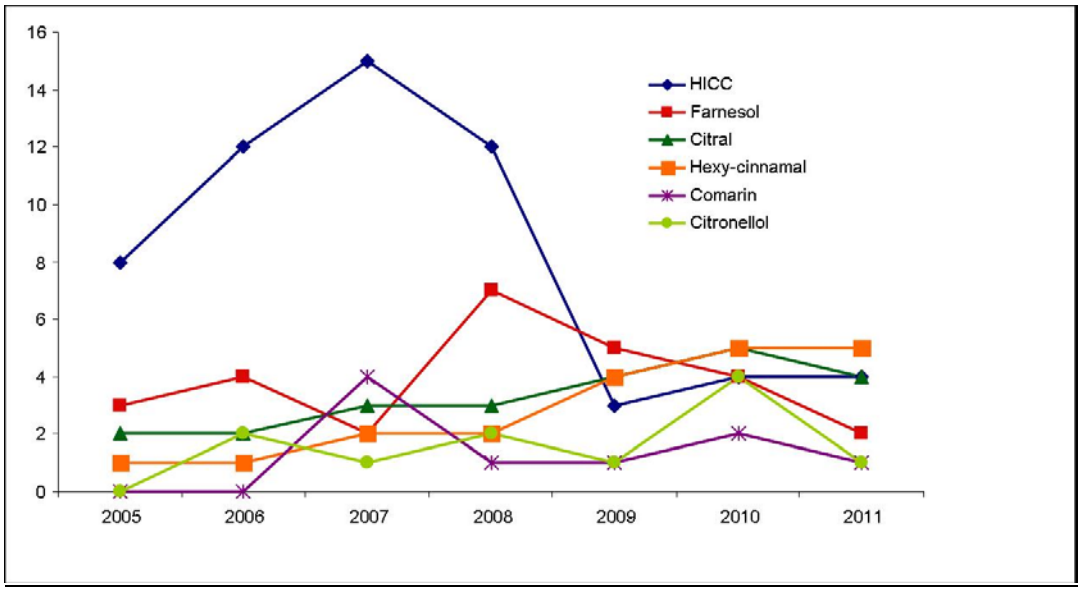

Figure 2. Evolution of the absolute numbers in frequency over the years of positive reactions to the different ingredients of fragrance mix 2 (2005-2011). HICC, *hydroxyisohexyl 3-cyclohexene carboxaldehyde*.

The associated positive tests observed with the different ingredients of FM1, MP and colophonium are given in Table 4 showing an association for all comparisons reported. When significant, the OR was computed. As an example, an OR of 915.09 (Table 4) means that the odds of a positive reaction to cinnamic alcohol is 915.0 times higher for patients with a positive reaction to cinnamal than for the non-cinnamal allergic patients.

Table 4. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the different fragrance mix 1 ingredients and *Myroxylon pereirae* and Colophonium

Allergens	**OR (95% CI)**
Cinnamic alcohol by Cinnamal	915.0 (428-1955)
Isoeugenol by Eugenol	140.6 (92-215)
Cinnamic alcohol by Amylcinnamaldehyde	98.7 (45-217)
Amylcinnamaldehyde by Hydroxycitronellal	56.4 (23-138)
Cinnamal by Amylcinnamaldehyde	55.1 (20-152)
Geraniol by Hydroxycitronellal	50.9 (24.18-107.5)
MP by Eugenol	46.9 (31-72)
Isoeugenol by Oakmoss	42.9 (30-62)
Eugenol by Cinnamic alcohol	38.3 (23-63)
Isoeugenol by Geraniol	33.3 (17-66)
Hydroxycitronellal by Oakmoss	32.5 (15-68)
Eugenol by Oakmoss	29.7 (19-46)
Isoeugenol by Hydroxycitronellal	28.7 (17-50)
Amylcinnamaldehyde by Oakmoss	28.5 (13-64)
Eugenol by Cinnamal	26.4 (13-54)

MP by Isoeugenol	25.4 (18-36)
Geraniol by Oakmoss	25.2 (16-39)
Cinnamic alcohol by Oakmoss	25.1 (16-39)
Cinnamal by Hydroxycitronellal	24.5 (11-56)
Cinnamic alcohol by Hydroxycitronellal	22 (12-42)
Geraniol by Eugenol	21.4 (9-49)
Geraniol by Cinnamal	17.4 (5-58)
Eugenol by Hydroxycitronellal	16.4 (8.21-33)
Isoeugenol by Cinnamic alcohol	14.4 (8-26)
Isoeugenol by Amylcinnamaldehyde	14.1 (5-41)
MP by Geraniol	13.6 (7-25)
MP by Cinnamic alcohol	13 (9-19)
MP by Cinnamal	12 (7-21)
Colophonium by Amylcinnamaldehyde	11.8 (5-26)
Cinnamal by Oakmoss	11.6 (6-24)
Isoeugenol by Cinnamal	10.5 (4-25)
MP by Oakmoss	9.3 (7-13)
Eugenol by Amylcinnamaldehyde	8.4 (2-36)
MP by Amylcinnamaldehyde	7.7 (3-17.22)
Colophony by Geraniol	6.9 (3-14)
MP by Hydroxycitronellal	6.4 (4-11)
Colophonium by Cinnamic alcohol	6 (4-10)
Colophonium by Eugenol	5.9 (4-9)
Colophonium by Oakmoss	5.8 (4-8)
Colophonium by Isoeugenol	4.4 (3-7)
Colophonium by Hydroxycitronellal	3.9 (2-7)
Colophonium by Cinnamal	3.5 (2-8)

DISCUSSION

We observed a frequency of 9.6 % positive reactions to FM 1, with its ingredients showing a fluctuating trend. *Evernia prunastri* was the most frequent allergen among them, with a remarkable peak in 1997 and 2000, the pattern of which could be attributed to the fashionable use of it in some cosmetics products, especially aftershaves and hydroalcoholics. Among the >100 constituents identified in *Evernia prunastri*, atranol and chloroatranol (degradation products of atronorin and chloroatranorin) figure as the most potent allergens (Johansen et al, 2006); in view of their extreme sensitization potencies, the Scientific Committee of Cosmetics (SCCP) came to the conclusion that both substances could not be used at all in cosmetics products (SCCP/0847/04) (Nardelli et al, 2009a). In addition, significant associations have earlier been observed for colophonium and *Evernia prunastri* (with *Evernia furfuracea* as a contaminant or substitute) because of the potential common presence of resin acids i.e. abietic acid and dehydroabietic acid, and their oxidized derivatives (Johansen et al, 2006). Moreover, a recent study by Uter *et al*. has identified two subgroups of *Evernia furfuracea*-sensitized patients, first those with sensitization to (oxidized) resin acids, as indicated by positive patch test reactions also to colophonium, and second those non-

sensitized to resin acids, but sensitized to common constituents of *Evernia prunastri* and *Evernia furfuracea* (Uter et al, 2012b). Furthermore, colophonium, MP and FM 1 also cross-react with other terpene-containing substances, among which essential oils but also compositae plants (Paulsen et al, 2005) (data on file).

The crude MP as such has not been used in perfumery since 1982, when de International Fragrance Association (IFRA) banned its use in fragrances. Since 1995 (Api et al, 2006), MP has been incorporated in fragrances as an extract or distillate, but patch testing showed that MP-sensitized subjects even react more strongly to these extracts. Exposure to MP, or to some of the single constituents, is apparently frequent, hence this 'historical' screening agent still remains important to detect fragrance sensitivity (Nardelli et al, 2008).

In the current study, FM 2 identified 6% of the subjects suffering from fragrance allergy, with 99 fragrance-allergic patients missed if only FM 1 had been tested. There was also a fluctuating trend in frequency to FM 2's ingredients, in which HICC was the most frequent allergen with an ascendance peak in 2007 but a descending one since 2009. In the case of hexyl cinnamal a moderate increase was observed in the last year. The high frequency could then be attributed to the common use of HICC in deodorant and other cosmetics product in EU, in rather high use concentrations, i.e. more than 3.0% in certain perfumes, which resulted in positive reactions in 1-2.7 of consecutively patch tested patients (Johansen et al, 1995-Larsen, 2002- Frosch et al, 1995- Frosch et al, 1999-Frosch et al, 2002). In North America, the prevalence was then found to be only 0.4%, which was considered to be mainly because of the presence of the ingredient in much lower concentrations in deodorants in the USA compared to the EU (Bruze et al, 2008). Recently, also Schnuch et al. (Schnuch et al, 2012) did observe a slight decrease of positive reactions to HICC, which may be the result of diminished exposure (used in less products and in lower concentration, the latter having been recommended by the International Fragrance Association (www.ifraorg.org) who, since 2009 provided guidance in this regard: 0.02 % for deodorants, lip products, and intimate wipes, and 0.2% for all other leave-on and rinse-off cosmetics (Api et al, 2010). However, in agreement with our results, in a recent study in which the 26 labelled fragrance ingredients were investigated, HICC still remained among the most frequent allergens (Heisterberg et al, 2011).

Significant associations between cinnamal and cinnamic alcohol, as well as with MP in which both are present, were observed (Table 4). The IFRA restricts the use concentration in cosmetic products of cinnamic alcohol to 0.1% for deodorant and lip products, whereas cinnamal has specific concentration restrictions to 0.02% for deodorant, lip products and 0.05% for the other cosmetic categories (43rd Amendment, IFRA standard 2009). It has been reported that cinnamal and cinnamic alcohol may generate a common hapten. This may be due either to their close chemical relationship, causing cross sensitization, or to their combined presence in many cosmetic products, leading to

concomitant sensitization. It is thought that cinnamic alcohol is a 'prohapten', transformed by alcohol dehydrogenase (ADH) in the skin to cinnamal. However, we found cinnamic alcohol to be a more important allergen than cinnamal, which could be explained by an additional sensitization mechanism (Buckley et al, 2006- Lepoittevin JP, personal communication 2012). Moreover, in agreement with the literature (Foti et al, 2011a-Devleeschouwer et al, 2008), cinnamyl alcohol contact allergy is very often related to ketoprofen photosensitization (which explains its recent slight increase in frequency): during the study period 48 out of 81 ketoprofen-positive patients were also tested to cinnamyl alcohol of whom 37 reacted positively, while only 6 out of 45 tested to cinnamal reacted to it; this even renders cinnamyl alcohol a potential marker for ketoprofen sensitization. Some authors (Girardin et al, 2006-Foti et al, 2011b-Stingeni et al, 2010) considered a possible cross-sensitization between 'an aldehyde function' in the ketoprofen molecule and cinnamic aldehyde, or formation of common metabolites (Girardin et al, 2006). However, a clear explanation for this phenomenon is still lacking.

Significant associations were also found between isoeugenol and eugenol, the former recognized as a strong sensitizer with its concentration in cosmetics being restricted by IFRA since 1998; in 2009 the IFRA standard (43rd Amendment) changed the restriction on isoeugenol to 0.01% for deodorants, lip products and 0.02% for hydroalcoholics, aftershaves, women facial and hand creams, intimate wipes and make up removers. On the other hand, eugenol is a much weaker sensitizer (Buckley et al, 2006). They do not in fact cross-react, despite their close chemical similarity and are both prohaptens that metabolise in the skin. According to our results the OR of simultaneous association was still important (140.6), but considerably lower than with the cinnamic derivates. Moreover, simultaneous reactions were found with MP that contains, beside other components, both eugenol and isoeugenol.

With regard to other significant associations (Table 4), no molecular explanation can be put forward, but concomitant sensitization seems likely; indeed, fragrance-allergic subjects tend to present multiple sensitivities.

A significant number of subjects (n=131) reacted negatively to the breakdown constituents but positively to FM 1, a phenomenon also noted by others (Uter et al, 2010-Johansen et al, 1995-Buckley et al, 2006). Possible explanations are:

- false-positive reaction to the mix
- each constituent acts as an irritant, which lowers the elicitation threshold for other allergens when tested in combination
- false-negative reactions to the individual constituents of the fragrance mix, because the concentrations tested are too low, or the skin penetration of FM 1 is increased by the

emulsifier sorbitan sesquioleate (also present though in *Evernia prunastri*) (Buckley et al, 2006)

- the existence of "compound allergy" to a combination of two or more ingredients of FM 1, i.e. by the formation of a new allergen (Johansen et al, 1995)
- a different evaporation potential of individual components compared to the mixture. The conditions under which the fragrance test preparations are stored when applied in test chambers may affect the diagnostics of fragrance contact allergy, resulting in false-negative reactions. As many fragrance compounds are volatile, application to the test chamber should be performed as close in time to the patch testing as possible and storage in a refrigerator is recommended (Mowitz et al, 2012)
- Cinnamal and cinnamyl alcohol were found (Mowitz et al, 2012) to be more stable when analysed as ingredients in FM 1 compared with when analysed in individual preparations.

A recent study by Bonefeld et al (Bonefeld et al, 2011) has indeed shown that mixtures have an increased potency in sensitization and elicitation of contact allergic reactions as compared with isolated fragrance allergens. These results may explain why fragrance allergy is a prevalent phenomenon in spite of the fact that many fragrance allergens are categorized as weak sensitizers. The authors consider that mixtures of fragrances, i.e. FM1 and FM 2, not only reflect normal exposures to perfumes, but also provide the optimal stimulus to the immune system, thereby expressing high diagnostic ability. Hence, in the light of the Bonefeld study, false-negative reactions to the ingredients certainly also account for this phenomenon (Bonefeld et al, 2011).

Limitations of the study

The main limitation of our study is that the individual ingredients of FM 1 and/or FM 2 were not tested in all subjects reacting to the mixes. This is because testing was only performed with the individual ingredients when the FM-sensitive patients were able to come back for later readings. Secondly, this was a single-centre study. Therefore, only a relatively low number of patients were positive to the individual ingredients of the mixture. However, we consider that the present study on fragrance materials expands on previous work from our unit (Nardelli et al, 2008); and provides a current perspective on the frequency of sensitization to FM 1 and 2 in the European baseline series and their ingredients, as well as simultaneous reactions observed to MP and colophonium.

CONCLUSIONS

Evernia prunastri and isoeugenol were found to be the most common single fragrance allergens in FM 1, whereas HICC and to a far less extend farnesol in FM 2.

Positive reactions to MP and FM 1 are frequently associated because they share common components, i.e eugenol, iso-eugenol, cinnamal, and cinnamic alcohol. The association between colophonium and FM 1 is partly due to the presence of resin acids in *Evernia prunastri,* but together with MP also to (oxidized) terpenes, being present in essential oils and Compositae plants as well.

A fluctuating trend in frequency of positive reactions has been observed with the individual ingredients of the mixes, with a recent slight increase for cinnamyl alcohol, being associated with ketoprofen photosensitization, and a descending trend, particularly for *Evernia prunastri* and HICC. This could be explained by concentration restrictions (e.g. HICC), fashionable fragrance composition changes, and last but not least by avoiding the use of the components legally required to be labelled on the cosmetic packaging by the perfume industry.

Chapter 10

Discussion and Future Perspectives

The first objective of this thesis was to determine the frequency of contact allergy to fragrance allergens. Our study, in which 6 to 9% of the patients investigated for contact allergy during a 15-year period reacted to the fragrance markers in the baseline series, is in agreement with the literature, according to which its frequency is between 6-14% in patients with contact eczema (de Groot, 2001-Buckley et al, 2003-Mortz et al, 2013). In the past decade, the prevalence of fragrance sensitivity, evaluated by testing only with FM I, varied considerably though. A percentage between 7 and 10 was observed among patch-tested patients in Europe (Schnuch *et al*, 2004- 1997-Temesvari *et al*, 2002-Bangha *et al*, 1996-Hasan *et al*, 2005- Buckley *et al*, 2000).

We observed a fluctuating, slightly decreasing trend in frequency of positive reactions and some of their ingredients over the years (Chapters 5 and 9). In Denmark, FM I reactions rose from 4.1% in 1985–1986 to 9.9% in 1997–1998 (Johansen *et al*, 2000), and a similar rising tendency was reported in Slovenia as well (Lunder *et al*, 2000). However, a decrease in FM I sensitivity was reported in several other studies (Schnuch *et al*, 2004- Larsen *et al*, 1998- Wohrl *et al*, 2001-Schnuch *et al*, 1997- Temesvari *et al*, 2002); for example, according to a European multi-centre survey, the fragrance sensitivity rate had decreased significantly from 13.1% in 1999 to 7.8% in 2002 (Bruynzeel *et al*, 2005). This was also observed in the USA, where the fragrance sensitivity rate diminished from 14% to 11.4% in the late 1990s (Marks *et al*, 1998-Marks *et al*, 2000) and, according to a more recent study, even down to 5.9% (Belsito *et al*, 2006). In the future, perhaps a further decrease in positive reactions to FM I allergens may be expected. Indeed, because of the labelling of 26 fragrance components by EU legislation (EU Directive, 2003), including those of FM I, as well as the outcome of recent dermatological studies on fragrance allergy and subsequent legal requirements [for example, reducing the concentration of isoeugenol (Tanaka *et al*, 2004)], the policy of some fragrance companies has changed. For instance, it has been shown that the new prestige perfumes contain less often or smaller amounts of fragrance chemicals included in FM I, compared with the older perfumes (Rastogi *et al*, 2003). Moreover, deodorants and domestic and occupational products quite often contain fragrance chemicals different from those present in FM I, such as limonene (Rastogi *et al*, 1998- Rastogi *et al*, 2001). This underlines the usefulness of new screening substances for the purpose of increasing the ability to diagnose fragrance allergy (Frosch *et al*, 2005 a-b). In 2005, Hasan et al. have shown that in contrast to FM I, there is a significant increase in the sensitivity rate to MP in recent years (Hasan *et al*, 2005), whereas the sensitivity rate to MP has been rather stable over the years in our study. Our studies, in agreement with others (Scheinman, 1996-Malanin *et al*, 1989-Malten *et al*, 1984) show that the majority of patients with fragrance sensitivity are women (Chapter 5), which reflects a greater exposure to fragranced cosmetics in them. Moreover, women with skin problems due to cosmetics might be more apt to consult a dermatologist. However, in view of an increasing usage also in men, the sex difference

may become less prominent in the future.

The frequency of fragrance allergy gradually increased from young to adult age, the highest peak being between 20 and 40 years (40%) for females and between 40 and 60 years (37.6%) for males, to decrease gradually again later on. The peak at that age in the female population could be explained by the greater personal use of fragranced products in young women, in particular. In males this could perhaps be explained by a different patient selection or a different consumer exposure. However, contact allergy to fragrance containing cosmetic products may also occur at an early age, as illustrated by our case report (see appendix): a severe allergic contact dermatitis occurred in the diaper area of an atopic one-year old baby-girl, due to contact allergy to both isoeugenol and parabens (the latter being weak allergens). The allergens were present in wipes and skin-care products especially designed for babies. Occlusion and barrier damage of the skin had facilitated skin sensitization. Hence, any child with persistent eczema, whatever the age, should be referred for patch testing.

The hands and face were the most commonly affected body sites, and significant associations between the type of fragrance and localizations were found (Chapter 5). Some fragrance allergens were specifically related to certain lesion localizations, for example, a significant association was found between HICC and axillae in females. The high frequency could then be attributed to the common use of HICC in deodorant and other cosmetics product in the EU, in rather high use concentrations, i.e. more than 3.0% in certain perfumes, which resulted in positive reactions in 1-2.7% of consecutively patch tested patients (Johansen et al, 1995-Larsen, 2002- Frosch et al, 1999-Frosch et al, 1999-Frosch et al, 2002). In North America, the prevalence was then found to be only 0.4%, which was considered to be mainly due to the presence of the ingredient in much lower concentrations in deodorants in the USA compared to the EU (Bruze et al, 2008). Recently, also Schnuch et al. (Schnuch et al, 2012) did observe a slight decrease of positive reactions to HICC, which may be the result of diminished exposure [used in less products and in lower concentration, the latter having been recommended by the International Fragrance Association (www.ifraorg.org) who, since 2009 provided guidance in this regard: 0.02 % for deodorants, lip products, and intimate wipes, and 0.2% for all other leave-on and rinse-off cosmetics (Api et al, 2010)]. However, in agreement with our results, in a recent study in which the 26 labelled fragrance ingredients were investigated, HICC still remained among the most frequent allergens (Heisterberg et al, 2011).

We also determined the frequency of positive reactions to the FM I and FM II individual components (Chapters 5 and 9). *Evernia prunastri* was the most frequent FM I allergen, as was illustrated in Chapter 9, with, according to the literature chloroatranol and atranol as the main allergens (Bernard et al, 2003). As part of our studies on FM I ingredients, we could show that polymer-based treatment of oak moss extract reduces the allergenic elicitation potential in

previously sensitized individuals only to a minor extent (Chapter 6). However, in view of more recent data and considering elicitation, the major sensitizers atranol and chloroatranol should be eliminated completely from *Evernia prunastri* (oak moss absolute) in order to be safe for consumers. A recent study by Mowitz et al, showed that the thin-layer chromatography patch test indicates the presence of several other sensitizers other than atranol and choroatranol in oak moss absolute (Mowitz et al, 2013).

The second most important allergen in FM I was isoeugenol, whereas HICC and to a far less extend farnesol in FM II. The use concentration of certain allergen, such as isoeugenol, has been restricted over the years. Besides EU regulations, the International Fragrance Research Association (IFRA, which provides recommendations to the perfume industry regarding the use of certain fragrance allergens, and which has guidelines on all of the 26 fragrance materials that need to be labelled), suggested in their guidelines that until May 1998, isoeugenol, for example, could safely be used at a level of 0.2% in consumer products (White et al, 1999), to be reduced in 1998 to 0.02% (IFRA, 2008). IFRA has since further restricted its use, and has revised the guideline on this material numerous times, most recently in 2008, when the use of isoeugenol was limited to 0.01% in category 1 (lip products) and category 2 (deodorants) (IFRA, 2008).

Positive reactions to MP and FM I were frequently associated because of common ingredients, i.e. eugenol, iso-eugenol, cinnamal, and cinnamyl alcohol. The association between colophonium and FM I, as has been said, is partly due to the presence of resin acids in *Evernia prunastri,* but together with MP also to (oxidized) terpenes, being present in essential oils and Compositae plants as well (Paulsen et al, 2005).

For some of the ingredients of the fragrance mixes, there was a slight increase in frequency of positive reactions over the years, for example, to cinnamyl alcohol, which could be linked to its relationship with ketoprofen photosensitization (Devleeschouwer et al, 2008). For others, such as *Evernia prunastri,* HICC, and iso-eugenol, a recent slightly descending trend was observed. This could be partially explained by concentration restrictions (e.g. as for HICC and iso-eugenol); fashion-related fragrance composition changes; and lastly by introducing required labelling of the components on the cosmetic package, that perhaps also encourages the fragrance industry to omit the ingredients to be labelled.

Another objective of this thesis was to identify the nature of fragrance allergens in specific cosmetic products. Labeling of the 26 individual fragrance ingredients has indeed proven to be very useful in order to identify specific allergens in contact dermatitis patients with cosmetic intolerance. Of the 30 causal fragrance allergens identified in this study (Chapter 8), 18 had been required to be labelled since March 2005. In the 15 different categories of cosmetic products that were analysed, geraniol and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), as well as limonene, in

particular, were the most frequent ingredients for which their presence could be confirmed in the causal specific cosmetic products; moreover, hydroxycitronellal and *Evernia prunastri* were the most frequent fragrance allergens suspected to be present in such products. The value of testing other allergens, such as FM II ingredients and limonene has been demonstrated as well: 40% of the reactions in 2009 were attributable to them. In addition, some essential oils were responsible for reactions to several specific cosmetic products, lavender oil being the most frequent. The fragrance allergens identified in this study most often correlated with eau de toilette/fine perfumes and deodorants, which contain higher concentrations of fragrance chemicals and in the latter case in an occluded area; but also skin-care products (moisturizers) were involved. Indeed, leave-on products are more likely to cause allergic contact dermatitis than rinse-off products, such as those used for cleansing.

However, managing patients with confirmed fragrance allergy often still proves difficult. Advising patients to use products without their specific fragrance allergen or to use those labelled as 'fragrance-free' will not necessary lead to avoidance of all contact with fragrance materials, owing to the possibility of cross-reactions to components that are not labelled or to discrepancies in the accepted definition of the term. Moreover, labelling may be misleading. This was illustrated by a case report (shown at the appendix of this manuscript): a widespread allergic contact dermatitis resulted from skin application of a supposedly non-scented moisturizing body lotion in a patient known to be fragrance allergic. However, this lotion contained an extract of '*Rosa centifolia*' (rose oil) that produced a positive patch test reaction, beside rose oil itself, farnesol, limonene and linalool; the latter two are indeed common ingredients of several essential oils (e.g. rose, lavender and geranium oils). Essential oils may, even at low concentrations, still elicit allergic reactions, especially in previously sensitized subjects as in this case. Therefore, statements such as 'non-scented' or 'fragrance- free' should not legally appear on the label of products that contain essential oils or other fragrance ingredients, albeit for other purposes. Furthermore, this case also underlines that, when diagnosing contact allergy in fragrance allergic-patients, it is important to test with the compounds that the individuals actually come into contact with.

Additionally, we determined which and how many topical pharmaceutical products in Belgium contained fragrances and examined the nature of the fragrance in products that played a role in iatrogenic ACD. In this regard, it is important to note that in Europe there is no legislation requiring labelling of individual fragrances in topical pharmaceutical products that are intended to be applied on diseased skin (Chapter 7). Indeed, 66 different fragrance components were identified in them and 10% of topical pharmaceutical products in Belgium were found to contain fragrance ingredients. Essential oils were present in several pharmaceutical topical products, particularly in those that were brought on the market several decades ago. Unfortunately, they were also found in

more recently marketed preparations, particularly those containing non-steroidal anti-inflammatory drugs that are widely used.

The fragrance-allergy markers in the baseline series did pick up most of the patients sensitized to fragrance allergens present in the 48 specific causal topical pharmaceutical products, and in those 38 different fragrance substances gave relevant positive reactions. This study also showed that beside the baseline series extended patch testing with the products used by the patient, along with the ingredients, is also needed because one fifth of them only reacted positively to an essential oil or to another fragrance allergen present. The possibility of testing all the components of a commercial product depends, of course, on the manufacturer's goodwill to provide all ingredients.

Besides their use as a pharmacologically active ingredient in some cases, the use of fragrances in topical pharmaceutical products is unnecessary. Indeed, since such products are applied on more vulnerable, and often diseased skin (wounds, leg ulcers, eczema, etc.), they thus constitute another important source of fragrance sensitization, which predisposes the patients to develop multiple sensitivities and to react also to fragrance-containing cosmetics (and other fragranced materials).

Conclusions

Fragrance contact allergy is more frequent in females than in males and, over the years, fluctuating trends (either increasing or decreasing) in frequency of patch-test results with the fragrance allergy-markers and some of their ingredients have been observed. The frequency of fragrance allergy gradually increased from young to adult age, to decrease gradually again later on, however, contact allergy to fragrance containing cosmetic products may also occur at an early age. Hands and face were the most commonly affected body sites, followed by legs, arms, and axillae; moreover, some fragrance allergens were specifically related to certain lesion localizations, for example, a significant association was found between HICC and axillae, particularly in females. Positive reactions to the different markers were frequently associated, because of common ingredients, for example with MP and FM I, or due to the presence of the same or cross-reacting oxidized terpenic compounds, as with colophonium, FM I, essential oils, and Compositae plants.
With regard to cosmetic products, geraniol and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), as well as limonene, in particular, were the most frequent ingredients for which their presence could be confirmed in the causal specific cosmetic products. As to topical pharmaceutical products in Belgium, 10% of them were found to contain 66 different fragrance components, among which several essential oils. Finally, our studies also showed that it is important to test not only with the fragrance allergy markers in the baseline series, or the ingredients of the fragrance mixes, but also with the products that the individuals actually come in contact with.

Future Perspectives

- There is no doubt that progress has been made in improving the safety standards of fragrances, however, the figures on adverse effects reported by dermatologists worldwide suggest that these are not sufficient. Materials with a low potential for adverse effects may escape the laboratory screening methods available today. Therefore, a need has arisen for "in silico" methods (QSRA studies) and in vitro methods involving human or animal cells to identify potentially hazardous allergens, but which should be correlated to human endpoints. This is particularly relevant in the context of prohibition of animal studies for cosmetic ingredients. However, in vitro tests for diagnostic purposes will not be widely/routinely available within the foreseeable future. This means that diagnostic patch-testing, a bioassay with inherent pitfalls, remains the "gold standard" and the only tool to diagnose contact allergy and evaluate patients with suspected allergic contact dermatitis.
- In order to improve primary prevention of fragrance contact allergy in the general population, and secondary prevention in those subjects already sensitized, many more fragrance allergens than those actually tested by clinicians should be investigated. Indeed, besides the hydroperoxides of limonene and linalool that have already shown to be important contact allergens in the clinic (Bråred Christensson et al, 2012, 2013) and that are now commercially available as patch test materials, other fragrance components, which are classified in the report of the SCCP as established contact allergens in humans, or fragrance sensitizers identified in LLNA essays or emerging from QSAR studies should be made available for patch testing. This would allow to evaluate their sensitizing potential in clinical settings, to monitor them and accordingly, to propose safety or, if necessary, legal measures (e.g. labelling, reducing use concentrations, or even eliminating certain components).
- From a clinical perspective it is important for the individual who is sensitized to one fragrance substance to know if they must also avoid chemically-related substances that cross-react with the original sensitizer. Cross-reactions between chemically-related substances have not been well studied so far.
- Dose-response data from clinical studies are available for only a few allergens. In order to establish individual safe levels, such data are required for all established allergens of concern.
- Most experimental studies are performed on individual fragrance ingredients, while exposure to allergens in cosmetic products concerns allergen mixtures, influencing the

risk of sensitization and elicitation. Better knowledge about “cocktail” effects would improve the basis of risk assessment and management.

- Finally, the impact of the behavior of fragrance substances, such as volatility, and autoxidation processes and skin penetration, and metabolism (bio-activation)- “prehaptens” and “prohaptens”- remains an interesting challenge.

Summary

This doctoral thesis aimed to describe the frequency of contact allergy to the fragrance-allergy markers as tested in the baseline (standard) series, i.e. Fragrance Mix I (FM I), Fragrance Mix II (FM II), along with their individual components, and *Myroxylon pereirae* (MP) and colophonium, and, in addition, to determine trends in frequency of contact allergy over the years. We sought to characterize the age, sex, and lesion location of the fragrance-allergic patients and to study the association between the positive tests observed with the different fragrance-allergy markers.

We also investigated whether the chemically modified oak moss absolute, treated by a polymer-based method and containing less amounts of the strongly sensitizing chloroatranol and atranol, still produced positive patch-test reactions in previously sensitized subjects.

We also wanted to identify the nature of the fragrance allergens in topical pharmaceutical products in Belgium and particularly those that had caused iatrogenic allergic contact dermatitis (ACD), as well as those responsible for ACD from specific cosmetic products in the patient population investigated.

In our studies, 6% to 9% of patients investigated for contact allergy reacted to the fragrance markers in the baseline series, with a fluctuating trend in frequency of positive reactions over the years. Our studies also showed that the majority of patients with fragrance sensitivity are women, which reflects a greater exposure to fragranced cosmetics in them.

The frequency of fragrance allergy gradually increased from young to adult age, the highest peak being between 20 and 40 years (40%) for females, and between 40 and 60 years (37.6%) for males, to decrease gradually again later on.

The hands and face were found to be the most commonly affected body sites, and significant associations between certain locations such as legs, arms, and axillae and specific fragrance allergens were observed; for example, a significant association was found between hydroxyisohexylcyclohexene carboxaldehyde (HICC) and axillae, at least in women.

We also determined the frequency of positive reactions to the FM I and FM II individual components. *Evernia prunastri* was the most frequent FM I allergen, followed by isoeugenol, whereas HICC, and to a far less extend farnesol in FM II.

Positive reactions to MP and FM I were frequently associated because of common components, i.e. eugenol, isoeugenol, cinnamal, and cinnamyl alcohol. The association between colophonium and FM I was partly due to the common presence of (oxidized) resin acids in *Evernia prunastri,* a FM I component, but together with MP also to (oxidized) terpenes, also present in essential oils and *Compositae (Asteraceae)* plants.

With regard to trends in frequency of positive reactions over the years for the ingredients of the fragrance mixes, there was a slight increase for cinnamyl alcohol, which could be linked to its

relationship with ketoprofen photosensitization. For others, such as *Evernia prunastri,* HICC, and isoeugenol, a recent slightly descending trend was observed. This could be partially explained by concentration restrictions in their use (e.g. as for HICC and isoeugenol), fashion-related fragrance composition changes, and lastly by the requirement of labeling of the components on the cosmetic package, which perhaps also encourages the fragrance industry to omit the ingredients to be labeled.

Another objective of this thesis was to identify the nature of fragrance allergens in specific cosmetic products. Of the 30 causal fragrance allergens identified in this study, 18 had been required to be labeled since March 2005. In the 15 different categories of cosmetic products that were found to be responsible for allergic contact dermatitis, geraniol and HICC, as well as limonene, in particular, were the most frequent allergens for which their presence could be confirmed in the causal specific cosmetic products; moreover, hydroxycitronellal and *Evernia prunastri* were the most frequent fragrance allergens suspected to be present in such products.

The value of patch testing with allergens other than FM I, MP, and colophonium, such as FM II ingredients and limonene was also demonstrated: 40% of the reactions in 2009 were attributable to the latter allergens. In addition, some essential oils were responsible for reactions to several specific cosmetic products, lavender oil being the most frequent. The fragrance allergens identified in this study most often correlated with eau de toilette/fine perfumes and deodorants, which contain higher concentrations of fragrance chemicals, and in the latter case also in an occluded area, but also skin-care products were involved. Indeed, leave-on products are more likely to cause allergic contact dermatitis than rinse-off products, such as those used for cleansing.

Additionally, we determined which and how many topical pharmaceutical products contain fragrance components and examined their nature in products that played a role in iatrogenic ACD. Ten percent of topical pharmaceutical products in Belgium were found to contain 66 different fragrance components, among which products marketed several decades ago but also more recently introduced topical drugs such as non-steroidal anti-inflammatory drugs (NSAID's). This predisposes the patients to develop multiple sensitivities and to react also to other fragrance-containing products.

In summary, in this thesis various aspect of fragrance contact allergy have been studied, the results of which may contribute to a better understanding on fragrance allergy, in general. Furthermore, our studies showed that it is important to test not only with the baseline series or the ingredients of the fragrance mixes, but also with the fragrance-containing products and compounds that the patients actually come in contact with.

Samenvatting

Dit doctoraal proefschrift heeft tot doel de frequentie van contactallergie voor parfumcomponenten, alsook trends in frequentie over de jaren heen, te onderzoeken. Dit gebeurde via analyse van patch-test resultaten bekomen met de parfummengsels, nl. "Fragrance Mix I" (FM I) en "Fragrance Mix II" (FM II), en hun respectievelijke inhoudsstoffen, en met Perubalsem ("Myroxylon pereirae") (MP) en colofonium, welke indicatoren zijn voor parfumallergie en aldus in de standaardreeks ter diagnosestelling getest worden. We karakteriseerden de leeftijd, het geslacht en de lokalisatie van de huidletsels van de parfum- allergische patiënten en bestudeerden geassocieerde positieve tests voor deze verschillende parfum-allergie markers.

We onderzochten ook of de chemisch gewijzigde eik mos ("*evernia prunastri*"), behandeld door een polymeer gebaseerde methode waardoor de concentratie van de sterk allergene inhoudsstoffen chlooratranol en atranol gereduceerd werd, nog steeds positieve patch- test reacties veroorzaakten in vooraf gesensibiliseerde patiënten.

We deden ook onderzoek naar de aard van de parfumallergenen in de in België op de markt gebrachte lokaal gebruikte farmaceutische producten, en in het bijzonder van deze die een rol hebben gespeeld in het veroorzaken van een iatrogeen-uitgelokte allergische contactdermatitis, alsook van deze die verantwoordelijk waren voor allergische contactdermatitis uitgelokt door cosmetica. Onze studies wezen uit dat 6 % tot 9 % van de onderzochte patiënten een contactallergie vertoonden voor de parfumallergie merkers in de standaardreeks, en dit met een variërende frequentie van positieve reacties over de jaren heen. De meerderheid van de patiënten met parfumallergie waren vrouwen, wat een grotere blootstelling aan geparfumeerde cosmetica weerspiegelt.

De frequentie van parfumallergie steeg geleidelijk aan van jonge tot volwassen leeftijd, met de hoogste piek tussen 20 en 40 jaar (40 %) voor vrouwen en tussen de 40 en 60 jaar (37,6 %) voor mannen, om geleidelijk weer af te nemen op latere leeftijd.

De handen en het gelaat bleken de meest aangetaste lichaamsdelen te zijn, waarbij significante associaties tussen lokalisaties zoals benen, armen en oksels met specifieke parfumallergenen geobserveerd werden; bijvoorbeeld een significant verband werd gevonden tussen oksels en hydroxyisohexylcyclohexeen carboxaldehyde (HICC), althans bij vrouwen.

Wat de frequentie van positieve reacties op de individuele componenten van FM- I en II FM betreft: *Evernia prunastri* was het meest voorkomend FM I allergeen, gevolgd door isoeugenol, terwijl HICC en in veel mindere mate farnesol dit waren van FM II.

Positieve reacties op MP en FM I werden vaak geassocieerd gezien vanwege gemeenschappelijke componenten, namelijk eugenol , isoeugenol, kaneelaldehyde (cinnamal) en kaneelalcohol (cinnamyl alcohol). De associatie tussen colofonium en FM I, met name *Evernia*

prunastri, wordt gedeeltelijk verklaard door de gemeenschappelijke aanwezigheid van (geoxideerde) harszuren, maar samen met MP ook door (geoxideerde) terpenen, tevens aanwezig in etherische oliën en *Compositae (Asteraceae)* planten.

Wat de frequentie van positieve reacties over de jaren heen voor sommige bestanddelen van de parfummengsels betreft, was er een lichte stijging ,voor cinnamyl alcohol, gekoppeld aan de relatie met ketoprofen fotosensibilisatie. Voor andere componenten, zoals *Evernia prunastri,* , HICC, en isoeugenol, werd recent een licht dalende trend waargenomen. Dit kan deels verklaard worden door reductie in gebruiksconcentratie (bijvoorbeeld voor HICC en isoeugenol), mode-gerelateerde veranderingen in samenstelling van de parfum, en ten slotte door de vereiste etikettering van deze componenten op de cosmetische verpakkingen, dat wellicht de parfumindustrie stimuleert om deze ingrediënten uit de samenstelling weg te laten.

Een ander doel van dit proefschrift was om de aard van allergene parfumcomponenten in specifieke cosmetische producten te identificeren. Van de 30 causale allergenen die in deze studie werden geïdentificeerd, waren er 18 die sinds maart 2005 dienden geëtiketteerd te worden. In de 15 verschillende categorieën van de specifieke cosmetische producten verantwoordelijk voor allergische contactdermatitis waren geraniol en HICC, en vooral limoneen de meest voorkomendeallergenen en hydroxycitronellal en *Evernia prunastri* de meest vermoedelijk aanwezige allergenen. Het nut van patch tests met andere allergenen dan FM I, MP en colofonium, zoals FM II ingrediënten en limoneen werd eveneens aangetoond: 40 % van de reacties in 2009 konden aan deze allergenen worden toegeschreven. Bovendien waren ook sommige essentiële oliën verantwoordelijk voor reacties op diverse specifieke cosmetische producten, met lavendelolie als de meest voorkomende. De parfumallergenen waren in deze studie het meest gecorreleerd met toiletwaters/ “fijne” parfums en deodorantia, die hogere concentraties van geurstoffen bevatten, en in het laatste geval ook in een afgesloten gebied;, ook huidverzorgingsproducten (moisturizers) waren betrokken. Inderdaad, zogenaamde “leave- on” producten (bedoeld om op de huid achter te blijven) geven meer kans op allergische huidreacties dan zogenaamde “rinse-off” producten die afgespoeld worden, zoals vb. huidreinigende middelen.

Daarnaast gingen we ook na welke en hoeveel lokaal aangebrachte farmaceutische producten in België geurstoffen bevatten en welke allergenen een oorzaak waren geweest van iatrogeen uitgelokte allergische contactdermatitis. Tien procent van de topische geneesmiddelen in België, waaronder deze in de handel gebracht tientallen jaren geleden, maar ook meer recent geïntroduceerde producten zoals niet-steroïdale anti-inflammatoire geneesmiddelen bevatten maar liefst 66 verschillende parfumcomponenten. Dit beschikt de patiënten voor om meerdere sensibilisaties te ontwikkelen en ook te reageren op andere parfum- bevattende producten.

Samenvattend, in dit proefschrift werden verschillende aspecten van contactallergie voor parfumcomponenten bestudeerd, wat kan bijdragen tot een beter begrip van parfumallergie. Verder is uit onze studies gebleken dat het niet alleen belangrijk is om te testen met de parfum merkers uit de standaardreeks, maar ook met de afzonderlijke bestanddelen van de parfummengsels, en niet in het minst met de parfum-bevattende producten en inhoudsstoffen waarmee de patiënten daadwerkelijk contact hebben.

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CurriculumVitae

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Andrea Nardelli was born and grew up in Reconquista, Argentina. She attended the San Jose School where she graduated with a secondary school degree oriented in Economics in 1990. She studied Medicine at the Universidad Nacional de Cordoba, Argentina and obtained her M.D. in 1997.

This was followed by a one-year residency in Internal Medicine at the Misericordia Hospital, and a three-year Dermatology residency at the Hospital Nacional de Clinicas in Cordoba, Argentina. In addition, from 1997 to 2002, she was a physician at a primary care centre in Córdoba, Argentina.

In 2002, she moved to Belgium for specialization program in dermatology mainly in Contact Allergy at the KULeuven. This triggered her interest in research in allergy and immunology, with a prolonged, fruitful and enjoyable stay in Leuven. A pre-doctoral programme was completed in 2005-2006 approved with greatest distinction of the jury. Since 2006, she has been a Doctoral Student at the Dermatology Department under the supervision of Prof. An Goossens. In the meanwhile she also attended to the out-patient general dermatology, phototherapy and laser therapy clinics to maintain her training in Dermatology. In 2010, she did another big move, this time to Canada. Since 2013 she has a post-doctoral position at Farncombe Institute of Digestive Health at McMaster University, in Hamilton where she performs research in the dermatological complications of therapy for inflammatory bowel disease.

She has co-authored 10 peer-reviewed papers including nine as first author

Other interests include history of art and philosophy. Her other passions are painting with which she tries to spend as much time as she possibly can and cooking Italian cuisine. She is married to Javier Ganame, cardiologist; has two adorable daughters, Magdalena and Amalia.